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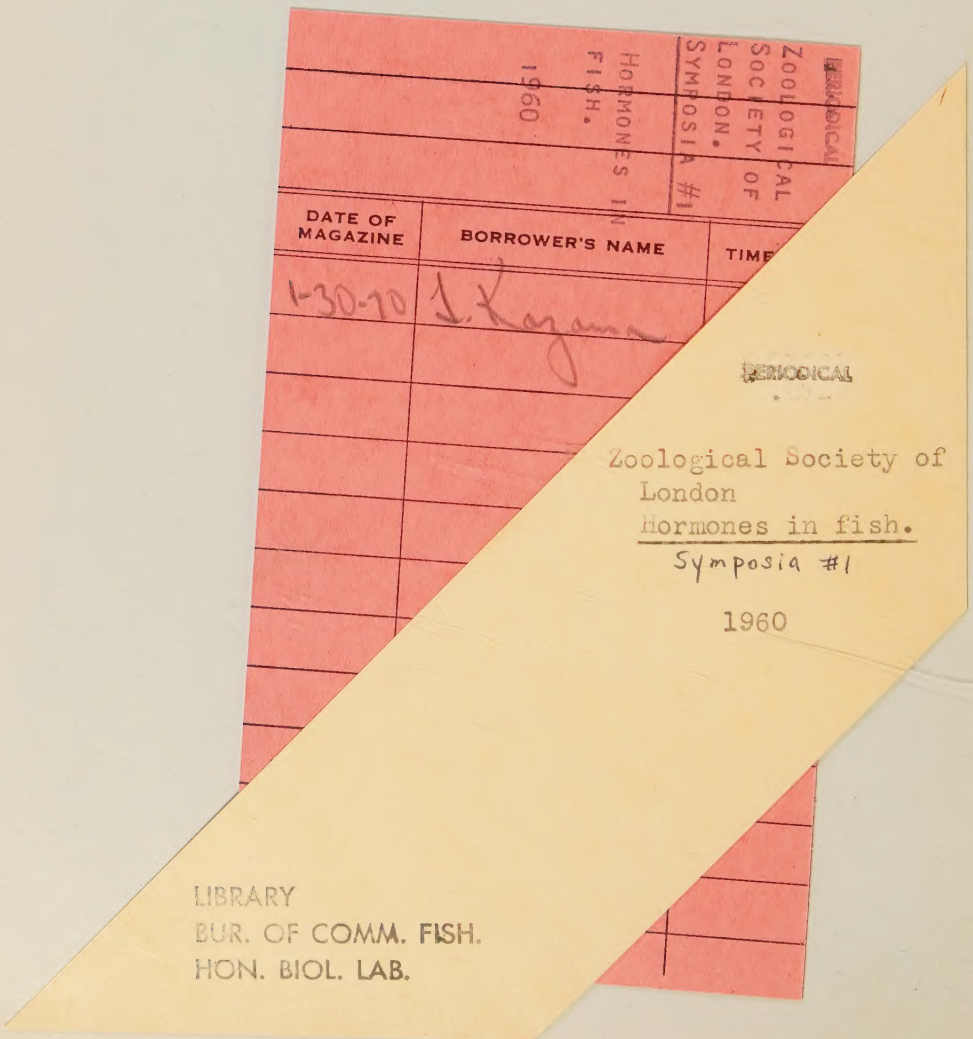
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HORMONES IN FISH

SYMPOSIA
of
THE ZOOLOGICAL SOCIETY OF LONDON

Number 1

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HORMONES IN FISH

Proceedings of a Symposium held at
The Zoological Society of London
Regent's Park, London, N.W.1
on 13th October 1959

Edited on behalf of
The Zoological Society of London by
I. CHESTER JONES

LONDON
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1960

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CONTRIBUTORS

AMOROSO, E. C.	Department of Physiology, Royal Veterinary College, London, N.W.1.
BAGGERMAN, BERTHA	Zoological Laboratory, University of Groningen, Haren (Gr.), Netherlands.
BALL, J. N.	Department of Zoology, University of Liverpool.
D'ANCONA, UMBERTO	University of Padua, Italy.
DODD, J. M.	Gatty Marine Laboratory, University of St. Andrews.
EVENNETT, P. J.	Gatty Marine Laboratory, University of St. Andrews.
GODDARD, C. K.	Gatty Marine Laboratory, University of St. Andrews.*
JONES, I. CHESTER	Department of Zoology, University of Sheffield.
MARSHALL. A. J.	Department of Biology, Monash University, Victoria, Australia.
MORRIS, R.	Department of Zoology, University of Nottingham.
PHILLIPS, J. G.	Department of Zoology, University of Sheffield.

* Present address. Department of Biological Sciences, University of New South Wales, Sydney, Australia.

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PREFACE

In April 1959 the Council of the Zoological Society of London approved a proposal that three whole-day Symposia should be held annually in addition to the eight Scientific Meetings at which papers on diverse zoological subjects are read and discussed. The Society has also inaugurated a new publication—" Symposia of the Zoological Society of London "—in which the papers delivered at each Symposium, as well as relevant discussion, are published in separate volumes.

The reasons for this new move were straightforward. Scientific knowledge is now growing so fast in volume and scope, that specialization, regrettable though it may be, has become inevitable. Over recent years the experience of many scientific societies has shown that Symposia of the kind now embarked on by the Zoological Society of London probably constitute the most rewarding and economical means not only of scientific communication, but also of focusing light from various angles on major issues of wide significance.

The object of the Symposia is to bring together workers who are engaged in research on different aspects of any branch of zoology where rapid progress is being made, to provide them with an opportunity of giving an up-to-date account of advances in their particular fields, and to discuss the various contributions in relation to each other.

The organisers of the Symposia, who act as editors of the published volume in which the proceedings are recorded, are responsible for arranging the discussion, for selecting main speakers, and for encouraging discussion. Each organiser is allowed to co-ordinate the aspects of the subject he wishes his team to develop, in order to sharpen points of difference and to reveal the major issues in the progress of the subjects being discussed.

At least one worker from abroad is invited to take part in each Symposium.

Speakers are generally limited to about twenty minutes for the oral delivery of their papers, but scripts for publication may be up to 8,000 words in length and may be accompanied by figures and plates.

The Symposia are open to Fellows of the Society and to all other interested scientific workers, and notices of forthcoming Symposia are sent to the heads of Departments of Zoology in the United Kingdom and, according to the subject, to the heads of other departments which are likely to be interested.

S. Zuckerman.

May, 1960

GENERAL PROBLEMS OF OSMOREGULATION WITH SPECIAL REFERENCE TO CYCLOSTOMES

BY

R. MORRIS

Department of Zoology, The University of Nottingham

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INTRODUCTION

There are a number of aims in presenting this paper. In the first place I intend to review some of the evidence which has now accumulated about the processes of salt and water balance in cyclostomes. Our knowledge of these mechanisms is incomplete as yet and I hope that this survey will draw attention to some of the difficulties and deficiencies. There is, however, sufficient evidence to see in outline how the most primitive of living vertebrates have managed to exist in environments which may be very different in ionic composition from their body fluids and it is worth comparing these processes with those which have been developed by some of the other lower vertebrate groups. The osmoregulatory mechanisms of many of the lower vertebrates have been worked out in detail and have been reviewed from time to time (Smith, 1932, 1953 ; Schlieper, 1930 ; Krogh, 1939 ; Prosser, 1950 ; Black, 1954). One of their main points of interest is that they have been used as evidence to support palaeonotological data concerning the type of environment in which the early vertebrates arose and I propose to examine some of these arguments in the light of our increased knowledge of cyclostome physiology.

Systematics recognizes two main cyclostome groups : the Myxinoidea and the Petromyzontidae. This division is strikingly borne out when one considers the mechanisms of salt and water balance in the two groups. Myxinoid blood is isotonic with sea water and, like the elasmobranchs, the myxinoids thus largely avoid osmotic difficulties. The lampreys on the other hand, maintain their blood ionic composition relatively constant at a different level from that of their environment and therefore have to cope with the same sort of osmotic problems which also face the marine and fresh water teleosts.

MYXINOIDEA

The myxinoids are a marine group, whose migrations extend at the most, to short seasonal movements within the sea (Meek, 1916). The majority of the early blood analyses show that the blood of both *Myxine glutinosa* and *Polistotrema stouti* is slightly hypertonic to the environment. The validity of some of these measurements is difficult to assess because recently, a careful study by McFarland & Munz (1958) has shown that *Polistotrema* blood is normally isotonic with the surrounding sea water and it only becomes hypertonic when the animal produces slime as a result of handling. Stress reactions of this type make myxinoids difficult experimental animals and this should be borne in mind when evaluating results.

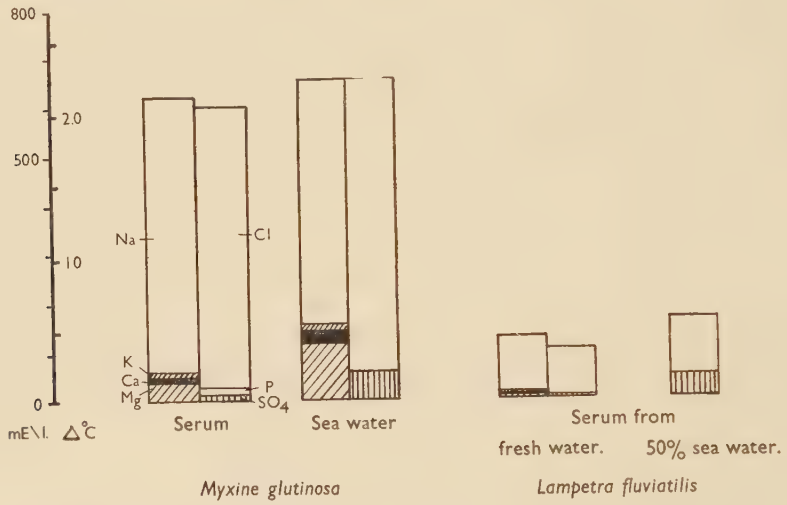

Fig. 1.—The ionic composition of the sera and environment of *Myxine glutinosa* and *Lampetra fluviatilis*. From Robertson (1954) and Morris (1958).

The ionic composition of *Myxine* serum (Fig. 1) has been determined by Smith (in Cole, 1940) and more recently by Robertson (1954), who has also reviewed the earlier literature on the subject. Both sets of analyses agree fairly well and they show that the concentration of divalent ions tends to be lower in the serum than in sea water. According to Robertson's figures, 44 per cent of the calcium, 67 per cent of the magnesium and 78 per cent of the sulphate are either excreted or excluded from the body. As to the monovalent ions ; serum chloride is somewhat lower and serum sodium rather higher than the environmental levels. The protein content of *Myxine* serum appears to be quite high, and Robertson suggests that this, together with some bicarbonate, makes up for most of the anionic deficiencies of the serum. Although as yet no one has dialysed serum against sea water, the distribution of monovalent ions obtained by Robertson suggests that the small differences which exist could perhaps be explained in terms of Donnan equilibrium. Robertson calculates that although the total ionic concentration of the serum

is about 2 per cent below that of sea water it exerts almost the same osmotic effect because the various ions are present in different proportions and some are more active osmotically than others. Borei (1935) claimed a rather high urea content for the blood of *Myxine* ; sufficient to have a noticeable osmotic effect. Needham (1938) based a theory for the origin of the myxinoids on these analyses, but the claims have not been substantiated either by the work of Smith or Robertson, who find very much lower amounts and Robertson believes that ions are responsible for 99·7 per cent of the osmotic concentration.

Little is known about the way in which salt and water balance is achieved. Morris (unpublished) obtained urine outputs of 1 to 8 ml./kg./day from *Myxine glutinosa*, a value which is of the same order as that given by most elasmobranchs (Black, 1954) and is in keeping with the osmotic balance between blood and environment. During the same series of experiments some animals swallowed quite large amounts of sea water, whilst others swallowed none at all and the fact that no measurable amount of water absorption took place from the gut to the blood, indicates that the gut fluid and blood must be isotonic. Analyses of the ionic composition of the urine are required to see if this is the route of calcium, magnesium and sulphate excretion. This certainly seems most likely, since the kidney is a well-developed glomerular structure whose short, tubular components and ureter are said to be cytologically equivalent to the proximal convoluted segment of the teleost kidneys (Gerard, 1942). The myxinoid kidney thus contains the same component structures and perhaps performs the same functions as the kidney of marine teleosts (Smith, 1930), that of excreting unwanted divalent ions and hence to some extent controlling ion balance.

There is little doubt that both *Myxine* and *Polistotrema* show typical poikilosmotic responses when transferred to concentrated sea water. Schmidt–Neilson & Schmidt–Neilson (1923) found that animals reached a new steady state when transferred to more concentrated sea water ($\Delta = 1{\cdot}85$ to 2·32° C.). The blood and the sea water osmotic pressure agreed completely after a few hours, and the changes were mainly effected by changes in the water content of the animals, showing that *Myxine* is more permeable to water than salts. McFarland & Munz (1958) confirmed that serum and sea water chloride and freezing point depression reach the same value when *Polistotrema* are transferred to different conditions, whilst Bond, *et al.* (1932), working on the same animal, found that the ratio of blood chloride to sea water chloride remained constant in various concentrations of sea water. This is in accordance with the view that the composition of the animal's sera follows that of the external environment fairly closely and that there is little attempt to keep the blood osmotic pressure constant.

It thus seems that the myxinoids have avoided osmotic problems because they remain isotonic with their environment under most circumstances. It would be interesting to know more about the limiting concentrations of sea water which the animals can stand and whether the tissues themselves are capable of any regulation under these circumstances, because the body fluids certainly fail to provide an effective buffer between environment and the cells themselves.

PETROMYZONTIDAE

Osmoregulation has been studied in three species of lamprey. *Petromyzon marinus*, the marine lamprey, and *Lampetra fluviatilis*, the river lamprey or lampern, have similar life histories. They start as Ammocoete larvae in fresh water and at metamorphosis, migrate seaward to parasitise fish. The larger marine lamprey makes its way to the open sea, whilst the lampern is thought to stay in estuaries and coastal waters where the salinity is lower (Bahr, 1952). As sexual maturity approaches, both of these animals ascend rivers, though at different times, and they eventually breed in fresh water. Although the dwarf brook lamprey, *Lampetra planeri* appears to have a similar life history and undergoes similar migrations, these are confined to fresh water and the adults never feed.

FRESH WATER OSMOREGULATION IN LAMPREYS

A number of investigations have been made into the mechanisms of osmoregulation in fresh water, particularly on adult animals during their spawning migration. Robertson (1954) has analysed the serum of river lampreys caught at this stage (Fig. 1) and his data indicates that the total ionic concentration is somewhat lower than that of fresh water teleosts. This observation agrees with measurements of the freezing point depression of serum obtained from migrating lampreys made by other workers (Dekhuyzen, 1904 ; Galloway, 1933 ; Morris, 1956 ; Hardisty, 1956). The levels of potassium, calcium and magnesium are of the same low order as in fresh water teleosts (3–5 mE/kg. water) and the chief difference between the two lies in the lower amount of sodium and chloride in lamprey blood. Hardisty (1956) has shown that the osmolar concentration of brook lamprey serum is somewhat lower than that of the river lamprey whilst Fontaine (1930) claims values for *Petromyzon marinus* which are higher than river lamprey serum. This comparison suggests that larger size may favour osmotic independence in lampreys perhaps because of the relatively smaller external surface for osmotic interchange in larger animals.

In view of the similarities of the blood composition and in the general organisation of the body between teleosts and lampreys, it is not surprising to find that they meet the same osmotic problems and appear to solve them in much the same way. There are indications from the urine output, however, that lampreys may take in more osmotic water than a fresh water teleost of corresponding weight. Urine outputs have been recorded which reach an upper limit of 360 ml./kg./day at 18° C. (Wikgren, 1953 ; Morris, 1956 ; Hardisty, 1956). This represents an extremely high urine output when compared with fresh water teleosts which appear to vary between 60 to 160 ml./kg./day (Black, 1954). Morris (1956) has criticised these results on the grounds that the techniques employed were likely to give rise to diuresis ; a phenomenon already well known in teleosts (Grafflin, 1931 ; Pitts, 1933). Values obtained by direct cannulation give much lower outputs which vary from 132 to 188 ml./kg./day, and these correspond well with the output recorded by Sawyer (from Black, 1954) of 159 ml./kg./day for *Petromyzon marinus*. The output is still high, however, when one considers the relatively

low osmotic pressure of lamprey serum, but it is difficult to decide whether this is due to a very high permeability of the external surfaces to water or to the increased area of permeable surfaces like the gills, because data on these specific points is lacking. Hardisty (1956) has calculated that the external surface of *Lampetra planeri* is twice as permeable as that of the lamprey, taking into account the disparity of size of the two animals, and his results indicate that the brook lamprey excretes more than its own weight in water per day.

Attempts have been made to analyse mechanisms of ion balance in fresh water lampreys. Just as in teleosts, there are three potential pathways for ion loss. Ions can diffuse through the gills and the skin and they are also lost to a lesser extent in the very dilute urine. Ion loss is compensated by a very efficient ion absorbing mechanism similar to that first demonstrated in the gills of teleosts by Krogh (1937). Wikgren (1953) showed that such a mechanism existed in lampreys. He found that ion uptake could be inhibited by low temperatures, and by keeping animals just above 0° C. for some time in very dilute solutions, he was able to estimate the rate of chloride loss by analysing the external solutions. After a period of ion depletion, the animals were returned to normal temperatures and their chloride uptake determined.

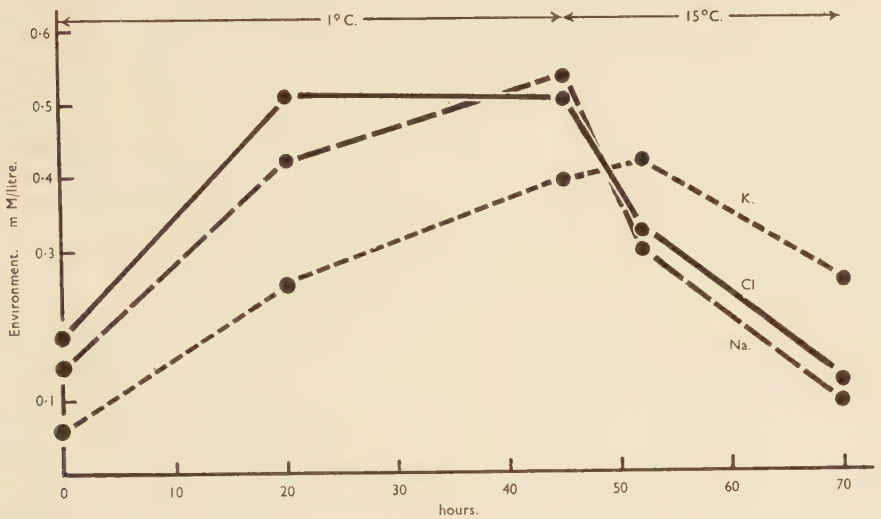

Fig. 2.—Change of ion balance in response to temperature change in an adult lampern. Weight 43·1 gm ; immersed in 300 ml of water. (Morris, 1957, unpublished.)

Fig. 2 shows an experiment of this type (Morris, unpublished) carried out on a river lamprey to show what happens to sodium, potassium and chloride ions. Using techniques of this type, Wikgren estimated maximum chloride losses of 24 mM Cl/kg. day which, according to Hardisty (1956) represent a loss of 70 per cent of the total body chloride per day. Values calculated from the present series of experiments (Fig. 2) are very much lower (4 mM Cl/kg./day) and these compare well with values obtained from *Lampetra planeri* by

Hardisty and with measurements made on other aquatic vertebrates (Krogh, 1937 ; Meyer, 1951 ; Barker-Jorgensen, *et al.*, 1947). Estimates of chloride uptake are similar to those of Wikgren (12 mM Cl/kg./day) provided that simultaneous chloride loss is not taken into account. If this is included, then uptakes amount to 16 mM Cl/kg./day which again corresponds fairly well with estimates obtained from other aquatic vertebrates. As in teleosts (Krogh, 1937), sodium and chloride are usually absorbed together (Fig. 2) and this may indicate that chloride absorption is secondary to that of sodium as in some other mechanisms of active transport (Ussing, 1949). The lamprey certainly appears to be unique in that it can absorb potassium from dilute solutions, presumably because the animal stops feeding during its spawning migration and therefore has to make up for the inevitable losses of potassium which occur in fresh water. In teleosts, it is assumed that potassium deficiencies can be made up from their diet.

Ionic loss from the kidney represents a small proportion of the total ionic loss according to Wikgren's measurements (0·1 to 1·2 mM Cl kg./day) and this argues for a very efficient ion absorbing mechanism in the kidney tubules; especially since the kidney is a glomerular one. The glomeruli and the kidney tubules are serially arranged along the length of the elongate kidney. Each nephron consists of a ciliated neck segment and a proximal tubule followed by a narrow distal tubule. Four or more similar units join into a common initial collecting duct (Regaud & Policard, 1901, 1902 a, b, c ; Morris, unpublished). The main point of interest here is the presence of a distal tubule ; a structure which is cytologically similar to that found in nearly all fresh water teleosts. Since the distal tubule is often absent in marine teleosts and the kidneys do not conserve ions in these animals (Smith, 1932), Grafflin (1937) holds that the distal tubule must be the site of ionic reabsorption in fresh water teleosts and this structure may serve the same function in the lamprey.

SEA WATER OSMOREGULATION IN LAMPREYS

The only available record from a lamprey taken from sea water suggests that marine osmoregulation is accomplished by a hypertonic mechanism. Burian (1910) quotes a value of —0·58° C. for a single specimen of *Petromyzon marinus* taken from the Mediterranean sea where the recorded freezing-point depression was —2·3° C. This value is very similar to that reported by Fontaine (1930) for the same species migrating into fresh water. Bahr (1952) working on *Lampetra fluviatilis* was able to capture sexually immature animals at Cuxhaven and reported that they began to suffer in concentrations of sea water above 65 per cent sea water, and this, together with field observations, led him to believe that most adult fluviatilis spend their life in the reduced salinity of estuarine waters.

Because migrating lampreys are fairly easily captured, attempts have been made to return these animals to sea water in order to study their osmotic responses. Fontaine (1930) working on *Petromyzon marinus*, and Galloway (1933) using *Lampetra fluviatilis*, both found that lampreys showed increased blood osmotic pressures following immersion in mildly hypertonic solutions, whilst sea water concentrations of 50 per cent and above were found to be

lethal in 24 hours. More recently Wikgren (1953) and Hardisty (1956) have also attempted to readapt migrating animals to sea water with little success. It therefore seems that an irreversible change must take place during the spawning migration when the fresh water mechanism replaces that responsible for sea water osmoregulation. Morris (1956) compared the marine osmotic responses of animals caught as early as possible in their spawning migration (fresh-run animals), with those of animals which were approaching maturity. The variability of the osmotic responses of fresh-run lamperns is perhaps most clearly shown in the results of experiments on ion balance. After immersion in 33 per cent sea water for a minimum period of 40 hours, the animals can be fitted into a graded series showing varying degrees of osmotic independence from their environment (Fig. 3). Some behave in much the same way as

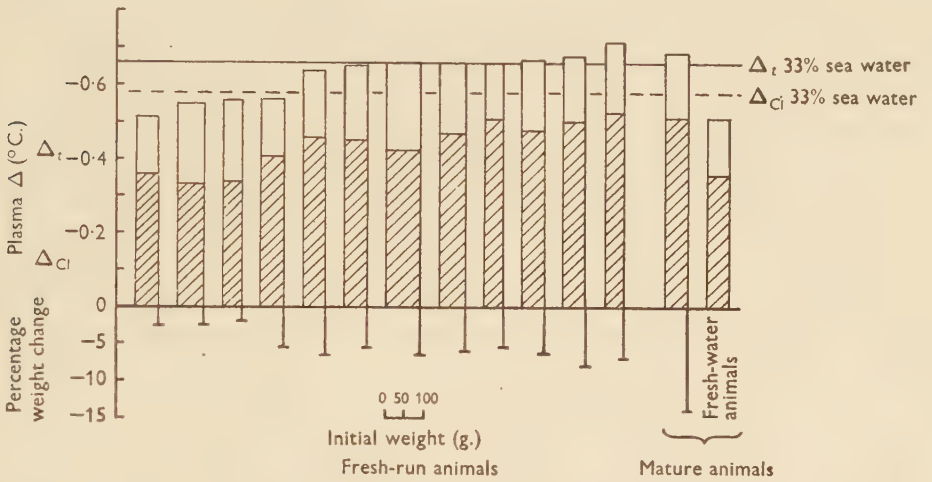

Fig. 3.—The osmotic responses of individual *L. fluviatilis* after abrupt immersion in 33 per cent sea water for a minimum period of 40 hr. (From Morris, 1956.)

maturing animals and would not survive prolonged immersion, whilst others keep their blood osmotic concentrations well below that of their environment and at the same time, manage to compensate for the osmotic loss of water. The most important facts which emerge from these experiments are that some animals can osmoregulate, whilst in others, the mechanism must be breaking down.

The teleosts, also osmoregulate in sea water and manage to maintain the osmotic concentration of their blood at about 1/3 the value of normal sea water. They thus face a similar problem to the lamprey osmoregulating in sea water. Smith (1930) was the first to show that they solved the problem of separating water from sea water by swallowing sea water, from which the animal absorbed a mixture of water and sodium chloride. The other salts, mainly a mixture of the divalent ions calcium, magnesium and sulphate, remained behind in the gut to be excreted later. Later, Keys (1931 a, b) was able to confirm Smith's observation that the excess sodium chloride which

entered the blood stream was excreted extrarenally by way of the gills against a concentration gradient. In this way, the animals obtain enough water to replace osmotic losses and produce sufficient urine to excrete the small amounts of divalent ions which enter the blood stream.

The river lamprey appears to employ a similar type of mechanism in hypertonic environments. So far, analyses have been restricted to a few fresh-run river lampreys which were capable of osmoregulating in 50 per cent sea water (Morris, 1958). These animals (Fig. 4) manage to keep their weight

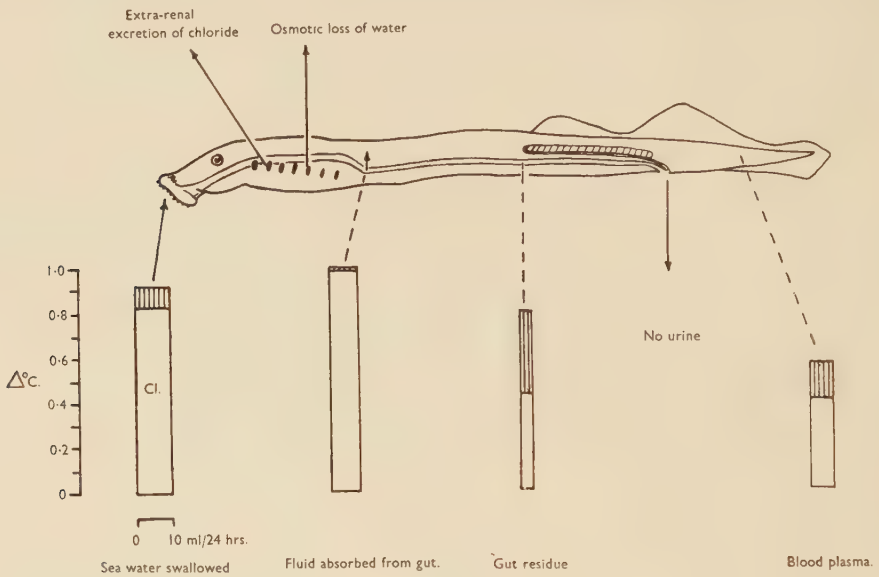

Fig. 4.—Data from a fresh-run lampern osmoregulating in 50 per cent sea water (data from Morris, 1958). Areas of the blocks are a direct measure of the amounts of ions involved.

constant by absorbing 75 per cent of the total amount of sea water which they swallow (10–20 ml./100 g./day), and since they produce little or no urine, nearly all the absorbed water must be lost by osmosis over permeable surfaces. Although 87 per cent of the chloride contained in the dilute sea water is also absorbed at the same time, the blood chloride remains at a very much lower level than that of the environment, so that in the absence of urine production one must assume that chloride is excreted extrarenally at a rate of 3 to 4 mM/100 g./day, presumably by way of the gills. There is also evidence that divalent ions like sulphate tend to remain behind in the gut due to a differential permeability of the gut wall to monovalent and divalent ions, and because of this very little enters the blood stream.

Some interesting facts come to light when we compare the mechanisms employed by the fresh-run lamprey and the teleosts. Qualitatively, they are almost identical, but from the quantitative point of view there are some marked differences. The rate of swallowing (200 ml./kg./day) and the amount of chloride excreted extra-renally (3–5 mM/kg./day) are roughly the same in

the lamprey and the eel, although the lamprey is subjected to a less steep osmotic gradient. It has to do approximately the same amount of osmotic work to be able to maintain itself in an osmotic gradient which is almost a third of the concentration experienced by the eel. One must conclude from this that the permeability to water or to water and salts must be very much higher in the migrating lamprey and this seems to be the chief difference between the lamprey and marine teleost in the present analyses. It is difficult to decide whether this high permeability is normal in lampreys or whether it is one of the factors which are partly responsible for the breakdown of the osmoregulating mechanism during the spawning migration. Bahr (1952) has shown that recently metamorphosed *fluviatilis* can only stand 65 per cent sea water and presumably high water permeability limits their activities to estuarine conditions. On the other hand, there is evidence from other sources that the water permeability increases during the course of the spawning migration (Morris, 1956), and the fact that some osmoregulating animals fail to produce urine in 50 per cent sea water, is perhaps an indication that this process is already under way in fresh-run animals.

The gills of marine teleosts contain numerous large acidophilic cells located in groups between their gill platelets. These cells were first discovered by Keys & Willmer (1932) who suggested that they might be responsible for the extra-renal excretion of monovalent ions. Since this time, quite a lot of information has been collected about these cells and the subject has been reviewed recently by Parry, *et al.* (1959). Similar cells are present in the gills of some fresh-run lampreys (Morris, 1957) which are almost identical in type, location and distribution to those described for teleosts by Copeland (1948) and Getman (1950). In the fresh-run lampern, however, there is a great deal of individual variation in the gill epithelium. Some have large numbers of chloride excretory cells, whilst in others there are signs that the gill epithelium is undergoing a marked histological change during which the chloride excretory cells disintegrate and disappear and new cells, much smaller than the original ones, take their place. Because these cells arise at a time when the marine mechanism of osmoregulation is being replaced by a fresh water one, it has been suggested that they are responsible for the active uptake of ions in fresh water and they have been tentatively called ' chloride uptake ' cells. The fact that similar cells also occur in ammocoete larvae and in adult *Lampetra planeri* adds more weight to this argument (Morris, unpublished). These observations are of interest in view of recent controversy about the functions of teleost chloride excretory cells. Bevelander (1935, 1936) maintained that the cells were only phases in mucus cell production, whilst Krogh (1939) doubted their osmoregulatory function. The more recent works of Liu (1942, 1944), Copeland (1948), Pettengill & Copeland (1948), Burns & Copeland (1950) and Getman (1950), strongly support the original hypothesis but provide no satisfactory direct evidence of chloride excretion. In the lamprey, mucus cells have quite a different type of structure from the chloride excretory cells. The mucus cells are small cells containing no mitochondria and they usually have a well-developed striated border. Chloride cells on the other hand are very much larger, contain large amounts of mitochondria

and have no striated border. Thus although both cell types develop from the same undifferentiated basal cells in the gill epithelium, there seems little possibility of functional relationship.

The factors concerned in the breakdown of the marine mechanism of the lampern have also been analysed (Morris, 1958) and their sequence is interesting because they provide information about the target organs which are affected during the anadromous migration. At least three separate processes change at the same time. The first of these processes is the increased water permeability of the external surface which was mentioned earlier. There is little doubt that fresh-run lampreys vary quite a lot in this respect and it could be that this increase forces them to seek environments where the osmotic stress is lessened. Fig. 5 shows the results of a series of experiments during which

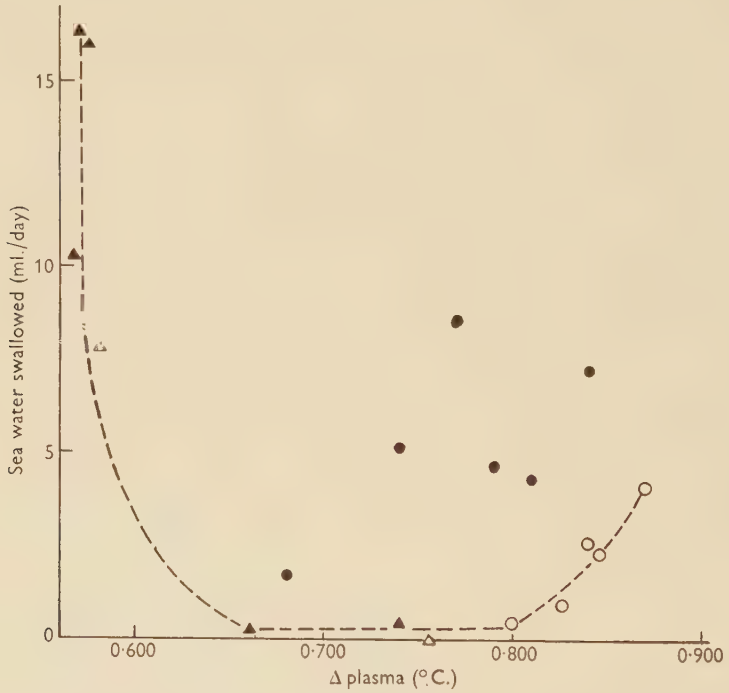

Fig. 5.—The relationship between swallowing capacity, chloride excretory cell assessment and plasma freezing-point depression (Δ) in fresh-run lamperns immersed in 50 per cent sea water. Chloride excretory cell assessment ▲, many ; ●, few ; Δ, very few ; ⊙, nil (from Morris, 1958).

the swallowing rate and the number of chloride excretory cells were assessed in a series of fresh-run animals immersed in 50 per cent sea water. It is only possible to consider detailed relationships in those cases where the number of excretory cells are shown as ' many ' or are absent altogether and these are joined by an interrupted line in the diagram. It is clear, however, that two processes are changing. In some animals, a reduction in swallowing rate is responsible for the failure to osmoregulate, in others, lack of chloride cells produces the same effects which become exaggerated when the animals absorb

ions from swallowed sea water. In yet another group, both breakdown processes go on at the same time. The reduction in swallowing capacity appears to be related to the degeneration of the alimentary tract which takes place when the animals cease feeding and there is a noticeable difference between individuals in the diameter of the gut lumen at the region of the pancreas. This analysis is also interesting from another point of view, because it gives an opportunity of studying what happens to a teleost-like marine osmoregulating mechanism when various parts of the mechanism are removed without surgical or chemical interference. Here, correlation between chloride excretory cell assessment and osmoregulatory ability argues very strongly for their role in monovalent ion excretion.

EVOLUTIONARY CONSIDERATIONS

Robertson (1957) has recently reviewed both the palaeontological and physiological evidence for the habitat of the early vertebrates and has come to the conclusion that the earliest vertebrates were marine animals which had to overcome the same sort of environmental stresses as present day vertebrates. He believes that the osmotic conditions in myxinoids are probably primitive and may be derived directly from marine chordate ancestors. These ideas conflict with the earlier views of Marshall & Smith (1930) who argued for a fresh-water origin for vertebrates and the development of the glomerular kidney as one of the consequences of life in fresh water. According to them the glomerulus was developed as an efficient means of excreting the osmotic load of water attracted by the high internal ion content of the early vertebrates. Robertson points out that *Myxine* has well-developed glomerular kidneys, and that these probably evolved in sea water and subsequently became a useful preadaptation to fresh water in other vertebrates. This explanation seems more likely viewed against the background of modern evolutionary theory and the weight of palaeontological and physiological evidence provided by Robertson and is strongly supported by the palaeontological findings of Denison (1956) and by the additional arguments put forward by White (1958).

Marshall & Smith (1930) and Smith (1932) have argued on both physiological and palaeontological grounds that the teleosts developed in fresh water and there seems no reason to change these views. They point out that glomerular development reaches its peak in fresh water forms, whilst in related marine forms the reversal of the osmotic gradient has resulted in a drastic reduction of urine output which has limited glomerular development. In a few cases this seems to have resulted in complete suppression of the glomerulus and the production of an aglomerular nephron. In terrestrial animals which faced similar problems of water shortage, the glomerular surface has either been cut down by including a core of connective tissue as in reptiles and birds, or a special water absorbing segment has been developed like that of mammals. Smith (1932) has collected data which shows that many lower vertebrates are incapable of producing a urine which is hypertonic to their blood and conserve water in this way, and the lamprey appears to show similar limitations (Morris, 1958). Smith argued that because of the limitations of the kidney in this

respect, and because the gut of lower vertebrates appears to be incapable of performing osmotic work, the marine teleosts developed the swallowing extra-renal excretory method of keeping their blood composition constant. If one accepts that the primitive fresh water mechanisms involved ion absorption from the gills and ion reabsorption by the kidney, then the changes that would be required to allow the animals to migrate seaward would be the ability to swallow sea water, an increased excretion of divalent ions by the kidney and a reversal of secretory polarity of the gill cells responsible for ion absorption. It is therefore interesting to note that Pettergill & Copeland (1948) claim such a reversal of polarity in the secretory cells of the gills of *Fundulus*.

Because of the remote relationship of the lampreys to the teleosts, one can only suppose that the similarity of their osmoregulatory processes are due to parallel evolution and that this has been brought about by similar environmental circumstances together with similar physiological limitations. Taking into account the fact that lampreys breed in fresh water, in addition to the evidence from their mechanisms of osmoregulation, the most probable course of evolution of their osmoregulatory mechanisms would thus seem to be that they came from marine ancestors which employed mechanisms similar to *Myxine*, they then developed their homoiosmotic mechanisms in fresh water and later added the marine mechanism.

If we accept Robertson's view that myxinoids owe their isotonic mechanism of ion and water balance to a marine ancestry then it follows that the vertebrate glomerulus must have been developed for reasons other than those of volume regulation by water excretion. Since fish appear to get rid of the greater part of their nitrogenous waste through their gills and body surface (Black, 1954), it seems unlikely that the glomerular kidney could have been developed for this purpose. There is evidence which suggests that the kidney of the Decapod Crustacea functions in much the same way as the vertebrate kidney, that is, as a filtration-reabsorption system (Ramsey, 1954). In this case there is no doubt that it was developed in animals which had a marine ancestry and that in some fresh water Crustacea an ion absorption segment has been added (Peters, 1935). Studies by Webb (1940) on *Carcinus* and by Robertson (1939) on *Cancer* indicate that the reason for the development of the kidney and the production of urine by these Decapod Crustacea is to help to control the ionic composition of the blood by reabsorbing sodium, potassium, calcium and chloride, and at the same time excrete unwanted magnesium and sulphate. This seems to be true of stenohaline marine decapods like *Maia* (Bialaszewic, 1932) and probably indicates that the crustacean kidney was developed primitively as an organ of ion balance and secondarily assumed the function of volume regulation in those animals which entered estuaries and fresh water habitats.

If it can be shown, as seems very likely, that the kidney of the myxinoids is also responsible for controlling ionic balance and we accept Robertson's ideas that they are primitively marine, then the vertebrate kidney would seem to have developed under very similar circumstances to those of the Decapod Crustacea and so far from the glomerulus being developed as a device for

controlling water balance as Marshall and Smith supposed, it seems much more likely to have been developed originally in sea water for the purposes of ionic balance, and later proved to be a useful preadaptation to fresh water, as Robertson suggested. In this new environment the kidney took over the added function of volume regulation after adding an additional segment for the reabsorption of ions.

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GENERAL DISCUSSION OF PAPER

Chairman : Professor Sir Solly Zuckerman, C.B., F.R.S.

F. G. T. Holliday : The evidence of a salt secretory function for the cells on the gills is of necessity circumstantial. Recent work by Dr G. Parry (Freshwater Fisheries Laboratory, London) and I on the teleost pseudobranch, an organ composed almost entirely of what have been called ' salt secretory ' cells, makes it clear that these cells are not in fact secreting salts. The anatomical disposition of the pseudobranch, both in relation to its blood supply and to the outside world (it is sunken below the epidermis in many fishes) precludes this function. Experiments on pseudobranchectomised fish (i.e. with the major site of the cells removed) indicate that ion regulation is not impaired, even under conditions of salinity which must be imposing considerable stress.

These and other experiments on the pseudobranch suggest that this organ (and therefore the cells of which it is composed) is intimately concerned with the colour reactions of fishes.

R. Morris : I think there are a number of points which need clarifying before one can disprove the osmoregulatory function of teleost and lamprey gill cells. In the first place one has to provide an alternative explanation for the circumstantial evidence which has been offered and I believe that this is quite strongly in favour of extra-renal excretion by these particular cells. With regard to the experimental evidence offered by Mr Holliday, Dr Parry and Mr Blaxter, the crucial point here seems to be whether or not the cells of the pseudobranch and those in the gill epithelium are identical. If they are identical, I am still doubtful whether pseudobranchectomy would be likely to bring about breakdown of the osmoregulatory mechanism in view of the large numbers of these cells in the gill eipthelium and the possibility of there being a considerable safety factor before osmoregulation would be affected.

P. M. J. Woodhead : My wife and I have been studying the effects of low temperatures on cod in the Arctic and some of the results parallel Dr Morris's observations on the changes in the number of ' chloride secretory cells ' found in the gills of lampreys in sea water and in fresh water. Below 2° C. the mechanism of osmoregulation appears to break down in the cod and the salt content of the blood (as shown by analyses of sodium, potassium, chloride and freezing point depression) increases. My wife has made an histological examination of the gills of these fish and finds that in cod from below 2° C., showing a high blood salt content, the ' chloride secretory cells ' have increased in numbers about four to sevenfold, and that the individual cells are larger, stain more deeply and show greater cytoplasmic granulation than normal. From their histological appearance we believe that the chloride-cells are in a state of increased secretory activity and, together with the increase in the number of these cells, we interpret this as being a compensatory response to reduce the higher salt content of the blood, the breakdown in the osmoregulatory mechanism occurring elsewhere.

R. Morris : I think these are most interesting observations which add more weight to the argument that these cells have an osmoregulatory function. I wonder, however, if the explanation of their increased number and the failure of the animals to osmoregulate might not be that the ionic output becomes reduced in response to low temperature and the increased number of cells represent an attempt to compensate for this ? I make this suggestion because there is no doubt that low temperature inhibits chloride uptake in the lamprey and this appears to be the cause of osmoregulatory breakdown in this case (Wikgren, 1954 ; Fig. 2 in this paper).

ADRENOCORTICOSTEROIDS IN FISH

BY

I. CHESTER JONES and J. G. PHILLIPS

Department of Zoology, University of Sheffield

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INTRODUCTION

Fish comprise a compendious and diverse group, containing members adapted to even the most bizarre niches of habitat. In their wide-ranging variety and in their economic importance, their study bears the possibilities of great interest and reward. For these reasons, fish have been by no means a neglected group. Few investigators, however, include them in the great body of endocrinological work now appearing as this is primarily directed towards the eutherian mammals. In the latter, well-recognised glands of internal secretion form chemical compounds, named hormones, and for which, in some cases, their structure has been elucidated and their mode of action tentatively delineated. Of necessity, therefore, the zoologist who contemplates the endocrinology of fish does so against the background of information gleaned from higher vertebrates. Thus in general it would be possible to suppose that either (i) hormones are not present in fish ; or (ii) hormones are present in fish but are of different constitution and type than those found in mammals ; or (iii) the hormones are similar to those of higher vertebrates. If this last proposition be the correct one, then it would be probable that the hormones of both fish and mammals would be involved in similar metabolic processes. As there are aspects of biological energy transformations which are common to many different types of organism, so may there well be unifying concepts of hormonal influence on cells which display the universal biological phenomena of exchange of materials between themselves and their environment (*cf.* Krebs & Kornberg, 1957). In this way, study in the field which we call Comparative Endocrinology might well produce results of general applicability. This paper emphasises such a unifying principle in consideration of one organ of internal secretion, namely the adrenal cortex.

GNATHOSTOMATOUS FISH

1. *Occurrence and types of corticosteroids*

In 1954 we set about seeking the types of adrenocortical secretion in representatives of different classes of vertebrates. It seemed a fair deduction from the riches of the older endocrinological literature (see Chester Jones,

1957a) that the third possibility noted above, namely the similarity of fish and mammalian corticosteroids, was the correct one. By 1957 we had found out enough to be able to say with a good degree of certainty that cortisol and/or corticosterone and occasionally cortisone, could be found in the blood of all animals from fish to man (Phillips & Chester Jones, 1957). One of us (Phillips) was fortunate enough to be able to continue investigations on these lines at Yale University. Thus by 1959 a wide range of elasmobranch and teleost fish had been examined and shown to carry in their peripheral blood concentrations of these corticosteroids of the same order as those found in

TABLE 1.—Adrenocorticosteroids in gnathostomatous fish

Expressed in μg/100 ml. plasma unless otherwise stated

Species	Common name	Ref	Male		Female	
			Cortisol	Cortico-sterone	Cortisol	Cortico-sterone
ELASMOBRANCHII						
Charcharinus milbertei	Brown shark	2			2.6	5.9
Charcharinus leucas	Bull shark	2	0.9	1.8	2.9	1.7
Charcharinus obscurus	Dusky shark	2			3.3	2.7
Charcharinus maculipinnis	Black tipped shark	2	2.9	0.8		
Galeocerdo cuvieri	Tiger shark	2			1.6	5.2
Dasyatus americana	Sting ray	2			4.3	4.0
Myliobatus freminvillii	Eagle ray	3	6.8	20.4		
Raja eglanteria	Ray	2			5.3	
Raja clavata	Ray	1	8.0 mixed male and female			
Scyliorhinus canicula	Dogfish	1	*2.5 mixed male and female			
ACTINOPTERYGII						
Gadus morrhua	Cod	1	*1.0 mixed male and female			
Pseudopleuronectes americanus	Flounder	4	16.0 mixed male and female			
Menticirrhus americanus	Southern kingfish	3	21.5 mixed male and female			
Pogonias cromis	Channel bass	3	2.0			
Catostomus comersoni	Short nosed sucker	2	44.0	7.7	20.0	18.5
Catostomus catostomus	Long nosed sucker	2	29.8	11.4	18.0	3.8
Cyprinus carpio	Carp	4	24.4		43.8	
Cyprinus carpio	Carp	3	24.8		31.3	
Fundulus heteroclitus	Killifish	3	15.2		8.2	
CHOANICHTHYES						
Protoperus annectens	Lungfish	1	15.0 mixed male and female			

* expressed as μg/100 ml. whole blood

References : 1. Phillips & Chester Jones (1957).
2. Phillips (1959).
3. Chester Jones, Phillips & Holmes (1959).
4. Bondy, Upton & Pickford (1957).

mammals (Chester Jones, *et al.*, 1959 ; Phillips, *et al.*, 1959). Tables 1, 2, 3 and 4 summarize our present knowledge. It should be emphasised that the concentrations given are those found in fish after capture and do not necessarily represent the resting levels. This point is returned to in § 3 below.

The finding that the main types of corticosteroids in fish are cortisol and corticosterone was extremely interesting. It still, however, left a lacuna in our knowledge of the physiological roles of the corticosteroids. For, as is well known, a broad division is made in eutherian mammals between those corticosteroids which influence aspects of carbohydrate metabolism, muscular energy and reactions to ' stress ' and those which control electrolyte movement. To the former belong cortisol, corticosterone and cortisone referred to uneuphonically but conveniently as glucocorticoids ; to the latter, aldosterone (Simpson & Tait, 1952), a mineralocorticoid. This dichotomy of physiological attributes is not a strict one, for cortisol, using this as one example of glucocorticoids, can alter not only patterns of electrolyte excretion but also those of water. Conversely aldosterone can show an effect in carbohydrate metabolism (Chester Jones, 1957 a).

Of course, the metabolic processes of the body are inter-dependent and any change from normal ionic balance is mirrored in multifarious cellular activities. Nevertheless, it is clear from mammalian work that though aldosterone is usually secreted in low concentrations compared with those of the glucocorticoids, it is an extremely potent steroid in its effect on sodium movement. Fish which must solve environmental hazards in terms of water and electrolytes, must be shown either to possess aldosterone or utilize other mechanisms, perhaps including the glucocorticoids, for these purposes.

TABLE 2.—Corticosteroids in plasma of spawning and spent male salmon (*O. nerka*) (Phillips *et al.*, 1959)

	Total steroid isolated (μg)	Corrected recovery*	μg/100 ml plasma
Cortisol	224.0	460.5	5.2
Cortisone	233.1	544.6	6.1
Corticosterone	276.0	644.9	7.3
Aldosterone	4.4	10.3	0.12
X_1	24.0	56.1	0.63
X_2	3.65	8.5	0.1
W_1	19.2	44.9	0.51

* It is presumed that steroids of similar polarity to cortisol would incur losses during chemical manipulations of a similar order to cortisol. The values have been accordingly adjusted but must be considered as approximate. Accurate recovery data is only available for cortisol (42.8 per cent) since this was the only labelled steroid added before extraction. Losses for corticosterone are probably greater than for cortisol. This figure is, therefore, minimal. Added labelled aldosterone (0.0016 μg) too small for subtraction. Aldosterone–16–H^3 added to purified extract (before chromatographing) showed a 50 per cent recovery.

The low concentration of aldosterone in the peripheral blood of the Eutheria made it likely, if this steroid were present in fish, that large quantities of fish blood would be required to establish its presence. To this end blood was collected from 364 spawning and spent male sockeye salmon (*Oncorhynchus nerka*) and these gave a pooled volume of plasma of 8,868 ml. Analysis by repeated paper chromatography and chemical identification showed the presence of aldosterone in a concentration of 0·12 μg/100 ml. plasma together with cortisol (5·2 μg/100 ml.), cortisone (6·1 μg/100 ml.) and corticosterone (7·3 μg/100 ml.), (Phillips, 1959 ; Phillips *et al.*, 1959). (Table 2.)

This identification of aldosterone has so far only been made from the blood of the salmon and it is inferential that it is universally present in fish. We shall bring forward other evidence, however, in support of the view that aldosterone will be found as a corticosteroid in teleosts (§ 2 below). Elasmobranchs have yet to be examined and offer a group of particular interest for their osmotic independence of seawater is gained by the retention of urea.

2. *The site of corticosteroid production*

We have shown that steroids of types similar to those found in the adrenal venous effluent of eutherian mammals are present in the peripheral blood of fish. It would be natural, therefore, to seek adrenocortical homologues in the lower vertebrates. In the Eutheria, the adrenal gland comprises two tissues, the medulla of sympathochromaffin cells, and the cortex of mesodermal origin. It is with the latter tissue that we are concerned, yet the varying involvement of cortical and medullary tissue displayed throughout the Vertebrata constitutes a fascinating story (see Chester Jones, 1957 a). It suffices here to point out that in teleosts, the presumptive homologue of the adrenocortical tissue of higher vertebrates lies in the head-kidney, often arranged around the cardinal veins. Chromaffin cells may or may not be found associated with this cortical tissue. In elasmobranchs, chromaffin and cortical tissue are completely separated and the latter occurs as discrete rods or oval glands lying between or against one or other of the posterior kidneys. Consideration of the changing form of the adrenal gland in the different classes of the gnathostomatous vertebrates, the elasmobranchs apart, reveal two major types of change :—(a) aggregation of chromaffin tissue from scattered islets to, finally, a mass of tissue arranged centrally in the gland about which the cortical tissue is set ; (b) diminution in the number of somites contributing to adrenocortical tissue formation concomitant with its movement away from the kidney. So that with the appearance of the metanephros in reptiles, the adrenal gland has become an encapsulated organ clearly separated from the kidney.

However much the gross anatomy of the adrenal gland differs in the various vertebrate classes, as between the interrenal of elasmobranchs, the anterior interrenal of teleosts, the suprarenal of Amphibia, and the adrenal glands or suprarenal capsules of reptiles, birds and mammals, there is histological similarity of its constituent tissues (Chester Jones, 1957 a). It is known that the corticosteroids arise from such tissue in the higher vertebrates

and the deduction can legitimately be made that these compounds found in the blood of fish had a similar origin.

Recent work goes some way in assuring us that this generalisation may be made. Phillips & Mulrow (1959 a) showed that incubation of the head-kidney of *Fundulus heteroclitus* in media containing added progesterone demonstrated that this tissue elaborated cortisol and aldosterone from this precursor. Nandi & Bern (1959 ; 1960) found that incubation of the head-kidneys of *Anoplopoma fimbria* (sablefish, marine) *Mugil cephalus* (mullet, euryhaline littoral) and *Tilapia mossambica* (cichlid, euryhaline fresh water) revealed the presence of cortisol and/or cortisone ; their yields of steroids were too small to allow of the identification of aldosterone. Supporting evidence for the widespread occurrence of aldosterone and cortisol in the lower vertebrates comes from recent work on the Amphibia (Carstensen, *et al.*, 1959).

In addition to the adrenocortical tissue lying in the head-kidney, some teleost fish possess small circumscribed bodies, named corpuscles of Stannius, lying on or embedded in the kidneys. The early claim that these corpuscles represented the adrenal gland of higher vertebrates was not substantiated and it was generally agreed that whatever function might eventually be ascribed to them it would not be adrenocortical in nature (Chester Jones, 1957 a). Recently, however, the problem has been re-opened. Rasquin (1956) suggested that the corpuscles of Stannius were concerned in osmoregulation and Vargas & Concha (1957 and pers. comm.) found a decrease in the concentration of sodium in the plasma of the teleost *Sicyases sanguineus* following their removal. More pertinently, Fontaine & Leloup-Hatey (1959) reported the extraction of significant amounts of corticosteroids from the corpuscles in salmon. However, Phillips & Mulrow (1959 b) were unable to show any capacity of the corpuscles in *Pseudopleuronectes americanus* to synthesize corticosteroids *in vitro* ; nor did Bondy (unpublished) and Ford (1959) find corticosteroids in extracts of corpuscles from carp and salmon. Although further experimental evidence is required before the ghost of the adrenocortical nature of the corpuscles of Stannius can be finally laid—or truly resurrected—their absence in many species seems to negative the idea of a universal function in fish.

3. *Functions of corticosteroids*

Although there is a similarity of secretory product from adrenocortical tissue throughout the Gnathostomata, the functions of such secretions are much less evident in lower vertebrates than they are in mammals. This is especially true when we consider their role in the metabolic processes which enable the organism to utilize stored foodstuffs for the release of energy. Krebs & Kornberg (*op. cit.*) have emphasized the unifying principles of energy transformations in all living organisms so that it would not be unexpected to find similar metabolic pathways for the conversion of fat, protein and carbohydrate in mammals and in fish. Undoubtedly, some of the factors which play an important part in food storage and energy release are found in all vertebrate classes. Thus it seems a reasonable suggestion that cortisol and corticosterone occurring in fish influence glycogenesis, gluconeogenesis,

TABLE 3.—Variation in adrenocorticosteroid levels

Species	Condition
Cyprinus carpio	Unstressed
	Slightly stressed
	a. Carried few metres
	b. Carried 50 metres
	Stressed by forced swimming
	a. For 30 mins.
	b. For 60 mins.
	c. For 120 mins.
Salmo salar	
Parr stage	
Smolt stage	
Adult	Anadromous migration
Adult	Spawning
Onchorhynchus nerka	
Female	Anadromous migration (300 miles)
Onchorhynchus nerka	
Male	Anadromous migration (640 miles)
	Spawning and spent
Onchorhynchus tschwytscha	
Sea-run Adult	
Adult	Anadromous migration
	a. 125 miles from sea
	b. 285 miles from sea
	Anadromous migration (325 miles from sea)
	Spawning and spent
Salmo gairdnerii	
Non migratory (immature)	
Sp. var. (mature)	
Migratory	Anadromous migration (118 miles from sea)

v = venous blood sample.

companying experimental and natural stresses

17-hydroxycorticosteroid μg/100 ml. plasma	Other steroids μg/100 ml. plasma	References
1.83±0.47		Hatey (1958) and personal communication
5.05±1.25		
8.95±3.31		
27.5±3.31		
28.1±9.15	corticosterone	
23.5±5.58	corticosterone	
19.6 (v) 12.7 (a)		Fontaine & Hatey (1954)
85.5 (v) 99.4 (a)		
50.2 (v) 31.4 (a) (male)		
45.3 (v) 34.7 (a) (female)		
31.7 (v) 21.2 (a) (male)		
30.6 (v) 25.7 (a) (female)		
54 (17 of cortisol, 37 of cortisone)		Idler *et al.*, (1959)
11.3 (5.2 of cortisol, 6.1 cortisone)	7.3 corticosterone 0.12 aldosterone 4 other Δ^4-3-ketones	Phillips *et al.*, (1959)
11.8 (mixed male and female)		Hane and Robertson (1959)
41.4±12 (males)		
49.0±21 (males)		
53.4±13 (females)		
29.0±11 (males caught in 1957)	Predominantly cortisol	
32.4±13 (males caught in 1958)		
88.5±35 (females caught in 1957)		
77.4±28 (females caught in 1958)		
2.7 (mixed males and females)		
10.0 (males)		
21.6±5 (males)		
34.1±15 (females)		

=arterial blood sample.

muscle activity and reactions to ' stress ' in this type of animal as these corticosteroids are known to do in mammals. Direct evidence, however, is largely lacking.

Support for the idea that there are similar pathways in carbohydrate metabolism may be gleaned from the remarkable uniformity in structure of the liver, with glycogen storage throughout the Gnathostomata (Elias, 1955). In the skate as in the rat, adrenalectomy results in a decrease in the amount of glycogen stored in the liver and a fall in blood sugar levels (Hartman, *et al.*, 1944). The converse, frequently demonstrated in mammals, has only been investigated in fish by Nace (1955, 1956) who showed that injections of a variety of corticosteroids increased the concentration of sugar in the blood of the toadfish (*Opsanus tau*).

Further similarities between fish and mammals can be seen in some of their reactions to muscular activity and to ' stress '. To endorse this view we have brought together in Table 3 information about the amount of corticosteroids in the blood of fish undergoing experimental stress and the stress occurring in nature as they perform prodigious feats of migration. It can be seen that in the carp, removal from water and transportation for varying distances results in increases in the level of 17-hydroxycorticosteroids in the blood beyond those found in quiescent—' unstressed '—fish. More stressful conditions, represented by forced swimming, are followed by larger increases though these are not displayed when the stress is continued (Leloup-Hatey, 1958). This might be due to the overstimulation of the adrenocortical tissue resulting in exhaustion of the interrenal in terms of its capacity to synthesize hormones. This is not an unusual phenomenon in fish and occurs apparently under natural conditions in the salmon.

In this fish the amounts of corticosteroids in the blood increase progressively from the values found for those in sea-run animals at 11·8 μg/100 ml. plasma to the high level of 70–80 μg at spawning, thence falling to lower levels during the postspawning period until death supervenes (Robertson, O. H., 1957 ; Hane & Robertson, 1959 ; Phillips *et al.*, 1959 ; Idler *et al.*, 1959 ; Robertson & Wexler, 1959). The increased levels of corticosteroids in the blood until spawning and their subsequent decline is accompanied by changes in the histological picture of the interrenal. During migration the adrenocortical tissue undergoes hyperplasia but once spawning is accomplished it shows atrophic changes which are consistent with the idea that continued pituitary secretion eventually causes overstimulation and then interrenal exhaustion (Robertson, O. H., 1957 ; Robertson & Wexler, 1959). It is interesting in this regard to compare the migratory steelhead trout with the non-migratory rainbow trout belonging to the same or nearly related species (Robertson, O. H., 1957 ; Hane & Robertson, 1959). The steelhead trout, during migration from the sea have two to three times the amount of 17-hydroxycorticosteroids in the plasma than do the rainbow trout remaining in their more constant habitat. Moreover, the interrenal of the steelhead trout undergoes hyperplasia during migration—as does that of the salmon—but this does not become atrophic during the postspawning period but many fish then die (Robertson & Wexler, 1959). The trend of these results is in keeping with

the earlier observations of Fontaine & Hatey (1954). These authors showed that the most active stages in the life-cycle of the salmon, that is during migration and at the smolt stage, are characterized by enhanced 17-hydroxy-corticosteroid production. On the other hand, they found that in the more quiescent parr stage the levels of these steroids were much lower.

It is important to remember that the salmon is not feeding when it is facing the severe stress of migration. During this period, therefore, the stored foodstuffs of the body are mobilised to supply the energy required for the considerable amount of muscular activity involved (Pentegov *et al.*, 1928 ; Black, 1958 ; Idler & Clemens, 1959). Thus, in the sockeye salmon by the time spawning has been completed, its protein reserves have been depleted by 30 per cent in the male and by 41 per cent in the female ; while fat reserves fell by 89 per cent in the male and 93 per cent in the female. This utilization of foodstuffs occurs during the phases when the amount of corticosteroids in the blood of the fish are particularly high, and it is reasonable to assume that these hormones influence the catabolic processes involved. It is possible, too, that these steroids are concerned in the mechanisms which allow of the efficient maintenance of the level of blood sugar during the spawning migration (Fontaine & Hatey, 1953). Further, in fish generally, muscular activity is often accompanied by increased blood sugar levels (Kiemeir, 1939 ; Secondat, 1950 ; Fontaine & Hatey, 1953). It is interesting to note, also, that active marine fish have higher blood sugar levels than do inactive marine and some freshwater forms have the highest values of all (Gray & Hall, 1930). But there is always the caveat that all sorts of extraneous factors cause changes in blood sugar levels, particularly, for example, oxygen deficiency which rapidly develops when fish are removed from the water.

We do not suppose that the corticosteroids are the only hormones, or indeed the most important hormones, in the utilization of foodstuffs in fish and the maintenance of adequate levels of blood sugar. We feel that the trend of the investigations so far, and this field is only now opening up, indicate that the adrenal hormones influence these processes. Clearly the participation of other hormones such as insulin, adrenaline, thyroxine and somatotrophin in metabolic transformations must be examined and this has yet to be done.

If, on the one hand, corticosteroids in fish aid the animal in its energy requirements, and this in a generally similar way to the position in mammals, we can, on the other hand, seek their activity in the realm of sodium and potassium movement across membranes. Though the nature of the environmental hazards of these aquatic animals has been constantly reiterated so that the marine forms battle with hypertonicity and the freshwater forms with hypotonicity of their environment in respect of body fluids, reiteration has not solved the mechanisms of their success. Teleosts are generally supposed to use both renal and extra-renal mechanisms for the purposes of osmoregulation. It is agreed that the gills are the main site of extra-renal mechanisms, though it is doubtful if histological identity of the cells involved is possible (*cf.* Morris, 1960). Gills in the invertebrates can be the site of

active transport, for example the Chinese mitten crab, *Eriocheir* (Koch, 1954). It would seem that cells have the basic property of selective ionic movement and it is not necessary to postulate hormones as being involved in these processes in the animal kingdom. Hormones, as we know them in the vertebrates, do not initiate processes but regulate them. But the fact is that aldosterone has been shown as present in a couple of species of teleost and just as we argued by analogy for cortisol function in fish from its action in Eutheria, so might we consider aldosterone to influence sodium movement in teleosts.

We know of only two pieces of work which have considered the possibility that the rate of movement of sodium ions across the gill membranes can be influenced by adrenocorticosteroids. One is by Sexton (1955) and remains an unpublished thesis, the other by Holmes (Chester Jones, *et al.*, 1959 ; Holmes, 1959). In neither was aldosterone used, cortisol and deoxycorticosterone were utilized as representatives of the secretions of the adrenal cortex which normally occur in fish (*cf.* Table 1).

Sexton used the goldfish (*Carassius auratus*) and found that the injection of 2·5 mg. DCA depressed the rate of sodium uptake by the gills, though it had no effect on sodium loss. Cortisone acetate, on the other hand, in the dose of 0·5 mg. had no effect on sodium uptake by, nor loss from, the goldfish gill either in normal fish or in those previously loaded with sodium chloride. Holmes investigated a euryhaline fish, the rainbow trout (*S. gairdnerii*) kept in freshwater but loaded with sodium chloride. Injection of 2 mg. DCA or of 10 mg. cortisol significantly enhanced the loss of sodium from the gills and also caused depression of sodium uptake from the outside water. It is probable that the dose of cortisone used by Sexton was not large enough to show the effect that he obtained with DCA and which Holmes obtained with both cortisol and DCA. It is clear in any case, that adrenocorticosteroids have an effect on sodium movement across fish gills and we may well anticipate that aldosterone would have an equal or even more potent action. The paradoxical action in the trout of both increased secretion and depressed uptake may well arise because of the problems presented to a euryhaline fish of remaining in freshwater yet coping with increased sodium chloride simulating the condition that would obtain in seawater. For it is presumed that the euryhaline fish in freshwater takes up sodium, and in seawater or in water of increased salinity, the animal actively excretes this ion through the gills. This dual process exists in the gills and can be influenced by corticosteroids. The position recalls the paradoxes in interpretation for the physiology of the mammalian nephron in which both sodium retention and sodium excretion can be obtained with the use of adrenal hormones (Chester Jones, 1957 a, b).

Another interesting point arises from Holmes' work namely that he found concomitant with increased rate of loss of sodium from the gills, a reduction of sodium loss from the kidneys under the influence of corticosteroids. It seems very likely that the similar adrenal hormones found in vertebrates are influencing similar basic metabolic pathways, and that essentially such diverse anatomical organs as the gill membranes, the skin of the frog and the nephron possess enzyme systems which respond in similar ways to the corticosteroids.

AGNATHOUS FISH

We have argued so far that it is reasonable to assume that, despite diversity of gross anatomical form, adrenocortical tissue produces similar types of secretions throughout the jawed vertebrates. It is possible that the most primitive type of fish, those without jaws—the Agnatha, though but distantly related to the Gnathostomata—might help to throw light on the evolutionary sequence of kidney function and correlative corticosteroid activities. Morris (1960, in this Symposium) has dealt with the general aspects of osmoregulation in cyclostomes and has considered the possibility of their marine origin. In this section we wish to examine the bearing that endocrinological considerations might have on these problems.

The Agnatha comprise two families, the Petromyzontia and the Myxinoidea, and representatives of each contain cortisol and corticosterone in their blood

TABLE 4.—Examination of *Amphioxus* and Cyclostomata for corticosteroids

CEPHALOCHORDATA		No corticosteroids found in 142 grams whole *Amphioxus*	
CYCLOSTOMATA		Type of corticosteroid	Levels (μg/100 ml. plasma)
PETROMYZONTIA			
Petromyzon marinus (Freshwater, landlocked lamprey of Lake Superior, N. America)			
	male	Cortisol	47
		Corticosterone	4
	female	Cortisol	28
		Corticosterone	5
MYXINOIDEA			
Polistotrema stouti (Pacific Hagfish)			
	mixed male and female	Cortisol	10
		Corticosterone	14

(Table 4). They thus fall into line, in this respect, with the whole of the vertebrate series. It would be anticipated that in cyclostomes these corticosteroids would be produced from adrenocortical cells similar in nature to those found in the Gnathostomata. It seems probable, though not absolutely sure, that in the Petromyzontia, at least, nests of adrenocortical cells extend much the length of the animal, set against the walls of the cardinal veins (Sterba, 1955 ; Chester Jones, 1957 a). The Myxinoidea have not been studied in this respect and this is particularly disappointing as they are unique in maintaining in their plasma concentrations of inorganic ions equivalent to those of the surrounding seawater (see Morris, *op. cit.*). Work is going on in this Department to fill the gaps in our knowledge and the main questions we wish to answer are (i) does aldosterone occur in the Myxinoidea? ; (ii) are there groups of true adrenocortical cells and do these produce aldosterone

and cortisol and corticosterone? ; (iii) what are the functions of these corticosteroids ? These questions cannot be answered at present by direct evidence but the pattern of adrenocortical secretion in vertebrates generally, allows us to suggest the kind of answers which we can expect to have in the near future.

If we return to a consideration of the Eutheria, the consensus is that one type of cell produces aldosterone together with some corticosterone. Such cells are found in one zone, the zona glomerulosa. Another type of cell is largely under the influence of the adrenocorticotrophin (ACTH) and synthesizes corticosterone, generally also cortisol and perhaps cortisone ; such cells are cast together in the zona fasciculata. A further main zone of the eutherian adrenal cortex, the zona reticularis surrounds the medulla (Ayres, *et al.*, 1956 ; Giroud, *et al.*, 1958). The relationship of the zones to each other and to the secretions they may elaborate has been reviewed recently (Chester Jones, 1957 a). It is not the purpose of this paper to re-examine the nature of the zones of the eutherian adrenal cortex but to take the undisputed knowledge about them into a consideration of the situation in fish.

It is possible to assert that aldosterone production is largely independent of ACTH whilst secretions of corticosterone and of cortisol in significant and varying amounts depends on this pituitary hormone. It can be stated, too, that the salmon, possessing adrenocortical tissue undifferentiated into zones, produces aldosterone, corticosterone, cortisol and relatively small amounts of cortisone ; and that these substances occur in the peripheral blood in concentrations of similar order to those found in mammals. Although we do not know the mechanisms controlling aldosterone secretion, we can deduce that cortisol and corticosterone are produced in response to an ACTH from the fish pituitary, variations in the secretory rate of which are reflected in the activity of the adrenal cortex. Supporting evidence has been discussed in the previous sections. Going a step further and contemplating the situation in the Myxinoidea we find that again the concentrations of cortisol and corticosterone in the blood are of the same order as those in salmon and mammals. A reasonable deduction would be that these corticosteroids are produced in response to the activity of an ACTH from the pituitary of the Myxinoidea. Any other hypothesis would not be in keeping with the evidence from the whole of the Vertebrata and it would require special pleading to suppose that the myxinoid pituitary was non-functional, so that its adrenal cells display an autonomous capacity for corticosteroid production.

This line of argument allows us to reach another conclusion. It seems likely the valid generalization can be made for all the Eutheria that the adrenal cortex of the hypophysectomized animal still synthesizes aldosterone while injections of ACTH can re-form those cells, and their metabolic pathways, involved in the production of one or more of the glucocorticoids (Chester Jones, 1957 a ; Giroud, *et al.*, 1958). This means that the cells producing aldosterone have enzyme systems capable of activity without hypophysial controlling mechanisms. If, then, we subscribe to the view that adrenocortical cells are similar throughout the vertebrates and if such cells in the myxinoids produce cortisol it would seem likely that the adrenocortical cells in these animals also produce aldosterone. This conclusion is susceptible to

experimental verification and a project to collect the large amounts of *Myxine* blood for the determination of aldosterone therein is under way in collaboration with Dr Day of the St. Andrews Marine Biological Station, New Brunswick.

The presence of typically vertebrate corticosteroids in Myxinoidea, and the distinct possibility that aldosterone will also be identified, are relevant considerations in a discussion of kidney function and the origin of vertebrates. The conclusions of Homer Smith on the physiological role of the kidney have always weighed the scales heavily in favour of the prevalent opinion that the origin of fish lay in freshwater. Romer, writing in 1955, said that the Romer–Grove paper favoured a freshwater origin but was not definitive: "The evidence for this same conclusion based on kidney structure is, however, of so substantial a nature that I am firmly convinced of its validity and hence find it difficult to believe that the palæontological evidence truly indicates the opposite conclusion". J. D. Robertson (1957), however, has done a considerable service in re-examining the problem and has pointed out that both the physiological and palæontological evidence can be marshalled to indicate that fish arose in the sea. From the point of view of the endocrinologist, two aspects of the function of the adrenal hormones, present throughout the vertebrates, are relevant. Firstly, the influence of corticosteroids on kidney function and secondly, on both intra-cellular and extra-cellular distribution of electrolytes. It may be supposed that in the vertebrate all the trunk segments are potentially capable of forming kidney tubules, though of course this potentiality is variously realised (Goodrich, 1958). We can expand this generalisation to say that the vertebrate body possesses a mesodermal component in the trunk somites which is potentially able to resolve itself into nephric, adrenocortical and gonadal components. Thus if the ancestral craniate can be said to have a complete set of segmental tubules throughout the whole trunk (Goodrich, 1958), then these would be accompanied by a similar extension of adrenocortical and gonadal tissue.

The expression of the adrenal cortex is directly correlated with the form of the kidney. This can be most clearly seen in the adult Tetrapoda (Chester Jones, 1957 a). Embryologically, of the 12 to 13 somites from the posterior end of the pronephros to the anterior side of the cloaca, the cortical tissue occupies 80 per cent in amphibia and there is a similar extension of the mesonephros. With the appearance of the metanephros, the adrenal cortex is compressed taking up 66 per cent of these somites in reptiles, 25–33 per cent in birds and 8·3 per cent in mammals. At the same time, the adrenal tissue separates from the kidney and forms a discrete encapsulated organ incorporating chromaffin cells. In the elasmobranchs, on the other hand, the mesonephros embraces the posterior segments and associated with it is the interrenal comprising solely adrenocortical tissue while in the teleosts it is the anterior segments which develop the adrenocortical tissue. The Agnatha seem to be nearest the proposed ancestral type with kidney tubules occurring down the length of the body and the nests of presumptive adrenocortical cells equally co-extensive. The nephros, then, is characteristic of the vertebrate body and equally characteristic is the adrenal cortex. It may well be that their appearance was necessarily concomitant in the true vertebrate.

We have emphasised that one major function of the corticosteroids is the regulation of ions at the kidney level. This can be regarded as a primary function and would thus be in keeping with Robertson's idea that the kidney first played a part in the mechanism of ionic regulation. There is no need, therefore, to place emphasis on the role of the kidney as an organ geared to the excretion of water, an opinion adduced constantly to substantiate the freshwater origin of fish (Smith, 1953). Rather the kidney can be regarded as the keeper of ionic and of acid-base balance. Furthermore, as Ramsay (1954) has pointed out, the maintenance of the internal medium is only half the story. The extra-cellular compartments of the body bathe the intracellular which are not simply in equilibrium but actively taking up some materials and are actively keeping others out. These properties are inherent in cells but in the vertebrates, certainly in the higher and probably in all, these processes require hormones, particularly the corticosteroids, for normal functioning.

Amphioxus has always been a puzzle for those seeking a vertebrate prototype. Smith (1953) felt forced to deny a relationship of *Amphioxus* to the vertebrates though it is remarkably similar to them in almost every basic feature (Romer, 1955). We have not found corticosteroids in a considerable sample of *Amphioxus* (Table 4) ; nor, if our hypothesis of the essential correlation of the vertebrate kidney with adrenocortical tissue be correct, would we expect to do so. Just as much as some invertebrates have developed a kidney filtration mechanism, perhaps concerned with ionic regulation (Ramsay, 1954) and others utilize nephridia, presumably for the excretion of nitrogenous waste, it is not really surprising that *Amphioxus*, a living representative of some of the pathways of Chordate evolution, should show vertebrate features coupled with possession of nephridia. Equally so would it be anticipated that its relatives in early times had vertebrate features plus nephrons, and were thus nearer to the main lines of eventual vertebrate evolution.

In brief, then, our speculation supposes that a mesodermic blastema of the true vertebrate, was characterized by its faculty of producing nephroi, adrenocortical and gonadal tissue. We suppose, further, as indeed Morris has suggested earlier in this Symposium, that the original role of the kidney was as an organ of ionic balance and to this we add the suggestion that the secretions of the adrenocortical tissue are essential for it to perform this function. Moreover, the regulation of ions which achieves a constant internal medium still demands activity by the cells for selection of ions present in this medium. Here, too, we consider that in the vertebrate, the corticosteroids are essential in this relationship, and were one physiological necessity to herald the evolution of the Vertebrata.

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FACTORS IN THE DIADROMOUS MIGRATIONS OF FISH

BY

BERTHA BAGGERMAN

Zoological Laboratory, University of Groningen, Haren (Gr.), Netherlands

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I. INTRODUCTION

In recent years studies on the causation of migration in fish have been mainly focused on the role of the endocrine system. Reviews on the subject have been given by Fontaine (1946, 1948, 1954, 1956 a), Fontaine & Koch (1950), Hoar (1951 a, 1953), Koch (1955), Baggerman (1957), Fage & Fontaine (1958). In the last few years new progress has been made in this field which

makes it worth while to once more survey the present status of our knowledge.

The current view on the causation of migration is often summarized in the statement that external factors are involved, in co-operation with changes in the neuro-endocrine system. However, this is a very vague generalization and when an attempt is made to analyse these factors in more detail, it becomes clear that the information on the subject is very fragmentary, which hampers its synthesis into a constructive theory.

The present review will be made on the basis of a schematical representation of possible factors involved (Fig. 1). In this figure migration is considered to consist of, most probably unspecialized, locomotion activities combined with special orientation components. Orientation is used here in the sense of " any reaction which guides the animal into its normal habitat, or into other situations which are of importance to it" (Fraenkel & Gunn, 1940). There is good evidence that migration only occurs when the animals are in the proper physiological condition (migration-disposition), and at the same time under the influence of appropriate external stimuli which act as ' releasers '. External factors which may act as ' releasers ' are temperature, light intensity, river discharge and meteorological factors. The physiological condition required for migration is thought to be mainly brought about by changes in endocrine systems, of which the thyroid, gonads, interrenal tissue and, of course, the pituitary seem to be the most important glands involved. There is evidence that the induction of this migration-disposition is controlled by external ' priming ' factors such as day length and temperature, and in addition by by endogenous rhythmic changes in endocrine activity.

The discussion will be limited to the analysis of causal factors involved in the induction of diadromous migrations. This restriction is made because so little experimental information is available concerning potamodromous and oceanodromous migrations (terminology of Meyers, 1949). Further, only observations and experiments made on migratory species of fish will be reviewed, since there is evidence that responses of resident and migratory species to e.g. changes in salinity and temperature can be quite different. Uncritical comparison of results obtained in experiments with migratory and non-migratory species has already led to much confusion in our thinking concerning the causation of migration. However, comparative studies on basic physiological processes in animals living in fresh and in sea water can be of great value for the understanding of processes and factors involved in migration, but a discussion of this matter lies outside the scope of the present review. Finally, although orientation has been mentioned as a component of migration in Fig. 1, factors involved in navigation during migration will be left out of consideration.

When there is evidence that certain processes and factors are involved in migration they have been made a component of the schematical representation given in Fig. 1. Relationships have been represented by solid arrows showing the direction of influence. Broken arrows indicate that there is evidence that no connection exists between two components. The possible way in which the two components of migration, locomotion and orientation, may be affected have been represented in Fig 1a. Only processes and factors which have been

Fig. 1

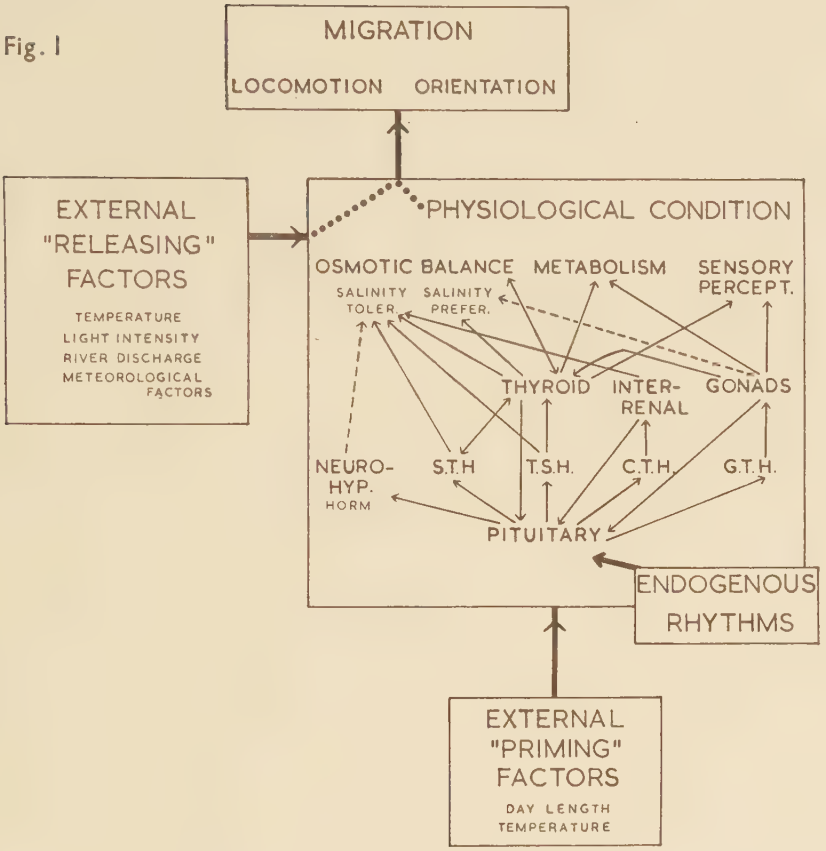

Fig. 1a

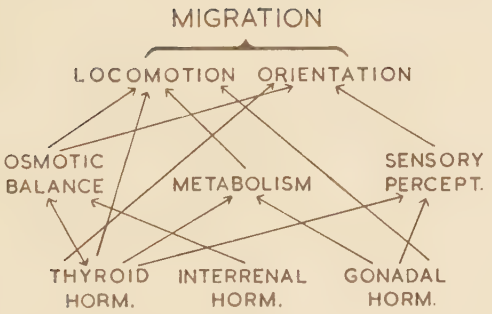

Fig. 1.—Schematical representation of factors involved in migration. For further explanation see text (p. 34).

Fig. 1a.—Detail of Fig. 1 illustrating the way in which various factors belonging to the physiological condition affect locomotion and orientation, the two components of migration.

shown to be involved in migration have been mentioned, but with increasing knowledge more and better defined components can be used to complete or revise the present picture. Frequent consultation of the figures will probably facilitate reading of this review.

II. THE PHYSIOLOGICAL CONDITION ASSOCIATED WITH MIGRATION

There is a good deal of information concerning the change in physiological condition which takes place at the time of migration. The first indications that a special condition is required before migration takes place came from field observations. Fontaine (1946, 1948, 1954, 1956 a) mentioned that in a population of young salmon (*Salmo salar* L.) living in a restricted part of a river and, therefore, exposed to the same external conditions, some animals migrate seaward when one year old, others when two years old, and a few even when they are three years of age. The only explanation for this difference

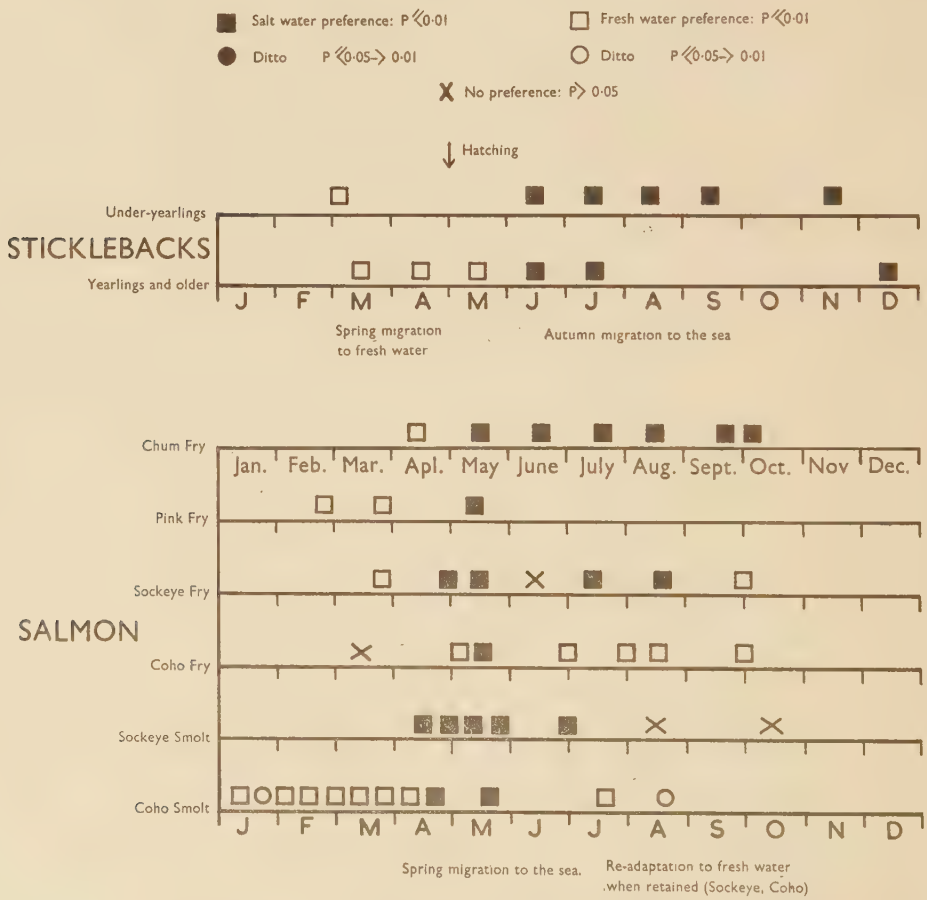
Salt water preference: P ≪ 0·01
Fresh water preference: P ≪ 0·01
Ditto P ≪ 0·05 -> 0·01
Ditto P ≪ 0·05 -> 0·01
No preference: P > 0·05
Hatching
Under-yearlings
STICKLEBACKS
Yearlings and older
J F M A M J J A S O N D
Spring migration to fresh water
Autumn migration to the sea
Chum Fry
Pink Fry
Sockeye Fry
SALMON
Coho Fry
Sockeye Smolt
Coho Smolt
Jan. Feb. Mar. Apl. May June July Aug. Sept. Oct. Nov Dec.
J F M A M J J A S O N D
Spring migration to the sea.
Re-adaptation to fresh water when retained (Sockeye, Coho)

Fig. 2.—Changes in salinity preference occurring at the beginning and end of the migration period in sticklebacks and juvenile Pacific salmon. Each symbol in this and the following figures represents the result of six tests or more.

in behaviour is that the animals differed internally. Fontaine (1954, 1956 a) and Hoar (1958) stated that the smoltification of juvenile salmon shortly before seaward migration is another example of a changed physiology, for it comprises profound morphological and biochemical changes.

Apart from these observations there is also experimental evidence that the onset and termination of migration is associated with a physiological change in the migrants. Baggerman (1957, 1959, 1960) used changes in salinity preference as criterion for the induction of this change in physiological condition. Only the main findings will be reported here. The experiments were carried out with the three-spined stickleback (*Gasterosteus aculeatus* L.) and with several species of juvenile Pacific salmon (*Oncorhynchus*).

When the salinity preference of sticklebacks and juvenile salmon was studied the year round it was found that the beginning and the end of the migration season were characterized by changes in salinity preference (Fig. 2). Sticklebacks migrate from the sea to fresh water early in the year and prefer fresh water at that time. At the end of the breeding season they migrate back to the sea and from that time until the next spring they have a preference for salt water. Under-yearling sticklebacks, hatched in spring, migrate to the sea in their first summer and autumn and at that time also prefer salt water. They mature the next spring and migrate to fresh water with the older animals and, like the latter, have a preference for fresh water at that time.

Similar results were obtained in four species of Pacific salmon, namely the chum (*O. keta*), pink (*O. gorbuscha*), sockeye (*O. nerka*) and coho (*O. kisutch Walb.*). Two species (chum and pink) migrate seaward as fry and do not survive in fresh water when retained beyond their normal migration time. Fig. 2 shows that after an initial short period of fresh water preference immediately after hatching, the animals begin to prefer salt water at the onset of migration and maintain this preference until they die in fresh water. Of the other two species only sockeye fry are occasionally found to move into the ocean in spring. At that time they also prefer salt water. Coho fry, on the other hand, never move actively into the ocean and in agreement with this it was found that they have only a very short period of salt water preference in spring, while there is evidence that, unlike the former three species, coho prefer only water of very low salinity. Both sockeye and coho salmon usually migrate seaward as smolt when one or more years of age. In Fig 2 it can be seen that in spring, smolts of both species have a strong salt water preference. Sockeye, as well as coho, survive in fresh water for many years when they do not reach the ocean, or have become landlocked. Sockeye salmon may even form successful breeding stocks in fresh water. In agreement with these observations is the fact that after the migration season has ended both species change preference and once again prefer fresh water. This indicates that they re-adapt to the fresh water environment in which they are retained and in which they are able to survive until the next migration period in the following spring. This is in sharp contrast with the behaviour of chum and pink salmon which are unable to re-adapt to fresh water. The results of these experiments confirm those of Houston (1957).

The main conclusion is that the beginning and the end of the migration season are marked by changes in salinity preference. Such changes are characteristic components of migration-disposition and indicate a change in the physiology of the fish. Under natural conditions it is very likely that together with a change in preference, changes in other processes involved in migration also take place. In this case the former change can be used as criterion for the induction of migration-disposition. However, it might be possible that under certain experimental conditions only salinity preference would be affected and not the other processes. In this case a change in preference does not reflect induction of migration-disposition, but only a change in one of its components.

III. THE NATURE OF CHANGES IN THE PHYSIOLOGICAL CONDITION AND THE CAUSAL FACTORS INVOLVED

In this section factors will be discussed which are involved in the induction of the physiological condition associated with migration. To study this problem one looks first for the processes which undergo changes at the time of migration and which may be considered to represent a component of this physiological condition, or a factor in its induction. There is good evidence that many endocrine glands, such as the thyroid, gonads, interrenal tissue and pituitary, undergo changes in secretory activity at the time of migration and it is likely that these systems may be involved at some stage or other. The first question which arises is whether these changes in endocrine activity are a causal factor in migration, or whether they are secondary phenomena brought about by a preceding induction of migration-disposition. A secondary phenomenon is thought to be induced by internal changes either directly, or by mediation of the external medium to which the animal is no longer adapted as a consequence of the internal changes. This problem is very complicated, particularly if one considers that a change in one endocrine system usually brings about a change in one or more other systems. The second question is in what way the endocrine systems may affect migration. It is possible that they may have a direct effect, and/or that they may induce changes in physiological processes which in their turn would affect migration (indirect influence). In the following sections the available information will be discussed from these points of view.

(1) *Endocrine glands influencing the physiological condition*

(a) *The thyroid gland*

Many species of diadromous fish show an increase in thyroid activity at migration time. Histological evidence for increased thyroid activity was found in the stickleback (Koch & Heuts, 1942), in the elver and eel (Sklower, 1930 ; von Hagen, 1936 ; Callamand & Fontaine, 1942; Fontaine & Callamand, 1943 ; Bernardi, 1948), in the sturgeon (Kalashnikow & Skadowskii, 1940, 1948, cited by Pickford & Atz, 1957), in the alewife and smelt (Hoar, 1952), in the smolt of Atlantic salmon (Hoar, 1939 ; Fontaine & Olivereau, 1947) and in fry and smolt of some species of Pacific salmon (Hoar & Bell, 1950). The histological findings indicating thyroid hyperactivity in the smolt of

Atlantic salmon were confirmed by experiments using thyroid uptake rates of radioiodine, and iodine contents of the blood as criteria (Fontaine, *et al.*, 1952). High values of blood iodine, indicating thyroid hyperactivity, were also found in adult Atlantic salmon and shad entering estuaries on their spawning migration (Fontaine & Leloup, 1950 a). Only a few cases have been reported in which no signs were found of thyroid hyperactivity at migration time. This was reported in the cyclostome fish *Petromyzon* entering fresh water on its spawning migration (Fontaine & Leloup, 1950 b). No histological signs of stimulation were reported in some specimens of seaward migrating smelt and alewife (Hoar, 1952) and in one species of Pacific salmon (Hoar & Bell, 1950). It should be added that in order to obtain conclusive evidence on thyroid activity different methods should be used simultaneously, as sometimes their results have been found to be contradictory (Fontaine, *et al.*, 1953).

Generally speaking one may conclude that most species of diadromous fish show increased thyroid activity at the time of migration. The question now arises as to whether the augmented thyroid activity is a causal factor in migration, or whether it is a secondary phenomenon brought about by a preceding induction of migration-disposition.

(i) *Thyroid activity and salinity preference*

One of the first attempts to answer the above question is to determine whether thyroid activity begins to increase prior to the onset of migration, or whether the increase takes place after migration has commenced. To obtain information on this point animals were caught in the field shortly before, during and after the migration season, and their salinity preference and thyroid activity determined. This was done in juveniles of two species of Pacific salmon, viz. the coho and sockeye. Full details on the procedures

TABLE 1.—Salinity preference and thyroid clearance in salmon parr and smolt

	COHO YEARLINGS			
	parr March 8–30	parr April 2–10	smolt May 1–7	smolt June 20–30
Salinity pref. (6 tests or more) Thyroid clearance	fresh $P \leqslant 0.01$ 0.07	fresh $P \leqslant 0.01$ 0.28	salt $P \leqslant 0.01$ 0.27	fresh $P \leqslant 0.05 -> 0.01$ 0.14
	SOCKEYE YEARLINGS			
		smolt April 2–10		smolt June 20–30
Salinity pref. (6 tests or more) Thyroid clearance	— —	salt $P \leqslant 0.01$ 0.22	— —	salt $P \leqslant 0.01$ 0.10

and results can be found in Baggerman (1960) and only the main findings will be reported here. The criterion for thyroid activity used was thyroid clearance of radioiodine from the blood. This is the per cent uptake of I^{131} by the thyroid during t hours, divided by the mean blood concentration of iodine during the same period. In the experiments described here this period was between 5 and 10 hours after injection of radioiodine. The higher the value of thyroid clearance, the more active the gland is supposed to be.

Coho yearlings were caught in March before the onset of seaward migration. They had distinct parr markings (sedentary phase) and salinity tests showed that they preferred fresh water, also indicating that migration-disposition had not yet been induced. Thyroid clearance in these animals was 0·07 (Table 1). At the beginning of April another group of parr were caught which also still preferred fresh water. However, thyroid clearance in these fish had increased about four times compared to that of the previous group and was 0·28. At the beginning of May a group of migrating coho smolt were caught at a counting fence on their way to the ocean. These fish had a strong preference for salt water and thyroid clearance was 0·27, about as high as in the previous group. The former animals were afterwards maintained in the laboratory and when tested at the end of June they had changed preference and once again preferred fresh water, indicating the end of the migration season and re-adaptation to fresh water (p. 37). In these animals thyroid clearance had dropped to 0·14.

The conclusion is that thyroid activity in coho yearlings begins to increase rapidly shortly before the onset of migration, indicating that this increase cannot be a secondary phenomenon caused by the induction of migration-disposition. On the contrary, it seems more likely that this increase might be a factor causing induction of migration.

The data given in Table 1 also show that thyroid activity decreases at the end of the migration period, but they are not conclusive as to whether this decrease may be a factor causing the end of the migration season, or whether it is a secondary effect. With respect to this problem data obtained in a similar experiment with sockeye smolt are more conclusive. Sockeye smolt were caught at the beginning of April when migrating out of a lake on their way to the ocean. They had a strong preference for salt water and thyroid clearance was 0·22 (Table 1). These animals were afterwards maintained in fresh water in the laboratory and when tested at the end of June they again preferred salt water, indicating that they were still in the migratory phase. However, thyroid clearance had already dropped to a value about half that in April, viz. 0·10. This indicates that the decrease in thyroid activity at the end of the migratory period is not a secondary phenomenon caused by the termination of migration, but seems to be a factor inducing this termination.

Apart from the above findings there is more evidence in favour of the hypothesis that the activity of the thyroid gland may be a causal factor in migration. These data were obtained from experiments based on the assumption that if this hypothesis were true, one would expect administration of thyroid hormone, or of thyroid inhibitors, to influence migration directly. This assumption was tested in sticklebacks and juvenile salmon using changes

in salinity preference as criterion for the induction of migration-disposition. Details on the procedure and results are given in Baggerman (1957, 1960) and only the main data will be given here.

(ii) *The effect of thyroxine and thyroid inhibitors on salinity preference and tolerance*

Table 2 shows that sticklebacks initially preferring salt water changed preference and preferred fresh water three to five days after immersion in synthetic thyroxine 1 : 10^9. This ties in with the finding of Koch & Heuts (1942) that thyroxine abolished salinity tolerance in sticklebacks living in sea water. On the other hand animals initially preferring fresh water began to prefer salt water three to five days after immersion in 0·036 per cent thiourea.

TABLE 2.—Changes in salinity preference in sticklebacks and juvenile salmon after administration of thyroxine and thiourea

	Salinity preference CONTROLS		Salinity preference THIOUREA treated		Salinity preference THYROXINE treated	
	P fresh	P salt	P fresh	P salt	P fresh	P salt
STICKLEBACKS						
October	—	⩽0.01	no experiments		⩽0.01	—
November	—	⩽0.01			⩽0.01	—
November	—	⩽0.05–>0.01			⩽0.01	—
December*	⩽0.01	—	—	⩽0.01	no experiments	
February*	⩽0.01	—	—	⩽0.01		
SALMON						
February	—	⩽0.01	⩽0.01	—	no experiments	
March	—	⩽0.01	⩽0.01	—	at these times	
April	—	⩽0.01	⩽0.01	—		
July	—	⩽0.01	⩽0.01	—		
July	—	⩽0.01	⩽0.01	—		

* These preferences were influenced by manipulation of the daily photoperiod

The conclusion is that in sticklebacks the amount of thyroid hormone in the blood influences their salinity preference. The results are in agreement with the fact that in spring, when the fish migrate to fresh water, they have a hyperactive thyroid gland (Koch & Heuts, 1942). Unfortunately, there is no information on the activity of the gland in autumn when the animals migrate seaward. It seems very likely that it will be low and this would be in keeping with the results of the thiourea experiments.

Similar experiments were carried out with juvenile Pacific salmon (Table 2). Coho yearlings were treated with thyroxine 1 : 10^6 and with thyroid inhibitors

(thiourea and thiouracil) in the concentration of 0·036 per cent. In animals initially preferring salt water thyroid inhibitors induced a fresh water preference. This is in agreement with the fact that coho yearlings before the onset of migration have a low thyroid activity and prefer fresh water (Table 1). Smith (1956) found that thiourea decreased salinity tolerance in *Salmo trutta*. However, administration of synthetic thyroxine had no influence on salinity preference in the above series of experiments with coho yearlings. It is possible that juvenile salmon do not respond as well as sticklebacks to synthetic hormone, but concentration differences may also have played a role. Hoar (1951 a) reported that treatment with thyroxine had no effect on salinity tolerance in coho yearlings. However, Fontaine & Baraduc (1954) found that treatment of parr of *Salmo salar* and *S. gairdnerii* with thyroxine was accompanied by an increase in salinity tolerance, and a similar finding was reported in *S. trutta* (Smith, 1956). Generally speaking it seems that treatment of juvenile salmon or trout with thyroxine may increase their salinity tolerance, and this would agree with the observation that thyroid activity is high at the onset of seaward migration (Table 1).

The general conclusion is that in sticklebacks as well as in juvenile salmon the level of thyroid hormone in the blood is able to induce changes in salinity tolerance and preference, and, thus, is probably also able to induce migration-disposition. This is a strong argument in favour of the view that thyroid activity is a causal factor in migration, and not a secondary phenomenon. Furthermore, it is clear that the level of thyroid hormone is also able to change the orientation of the animals towards fresh and salt water, which might be a component of the orientation complex involved in migration. However, there is no evidence as yet that this reaction may be used in navigation during migration. It is interesting to notice that thyroid hormone has opposite effects in sticklebacks and in salmon. This ties in with the observations that in spring, when thyroid activity is high in both species, sticklebacks migrate to fresh water, whereas juvenile salmon migrate seaward. We shall return to this point later.

(iii) *Factors influencing thyroid activity at migration time*

Various objections have been raised against the hypothesis that the activity of the thyroid gland is a causal factor in the induction of migration. These objections are mainly based on observations showing that at the time of migration many physiological and morphological processes undergo changes, which may be reflected in a change in thyroid activity. This makes it very difficult to ascertain whether thyroid activity increased primarily, or as a consequence of these changes. The most important factors which may influence thyroid activity at the time of migration will be discussed below.

Changes in osmotic balance

Changes in osmoregulation have been found in most species of diadromous fish at the time of migration, namely in lampreys, sturgeons, sticklebacks, eels, salmon and shad. The literature has been reviewed by Fontaine & Koch

(1950), Hoar (1951 a, 1953), Black (1951, 1957), Pickford & Atz (1957), Fage & Fontaine (1958). These changes in osmoregulation can be regarded as osmotic disturbances, since the fish are no longer properly adapted to their environment, although it does not necessarily kill them. As these changes in osmoregulation are correlated with the increase in thyroid activity occurring at the onset of migration, the question arises whether thyroid activity induces the osmotic disorder, or whether it is a consequence of the latter. Observations that thyroid activity becomes abnormally high when seaward migrating fish become landlocked (Hoar & Bell, 1950 ; Hoar, 1952) may indicate that ' stress ', caused by osmotic imbalance, increases the demands for thyroid hormone and, thus, might be the cause of thyroid hyperactivity at migration time (Hoar, 1951 a, 1953, 1958). In the salinity tests a preference for, e.g. salt water when the animals are kept in fresh water, may also reflect a change in osmoregulation, although a causal relationship between these phenomena has not yet been proven. However, supposing that a change in salinity preference reflects osmotic disorder, then the data given in Table 1 offer a strong argument against the view that osmotic stress would be the only cause of the increase in thyroid activity at migration time. For it can be seen that thyroid activity increased *before* the change in preference from fresh to salt took place, thus, before osmotic stress could play a role. Again, thyroid activity decreased *before* the animals changed preference from salt to fresh water at the end of the migration season and, thus, were still under stress for they were retained in fresh water. Therefore, although it cannot be denied that changes in osmotic balance may increase thyroid activity during the migration period, it cannot be the cause of the increase in thyroid activity which occurs in juvenile salmon prior to the onset of migration.

Metamorphosis

Another factor which may influence thyroid activity at the onset of migration is the concomitant occurrence of metamorphosis. The latter has been found in a number of diadromous species of fish, e.g. in the elver and eel, and in Salmonidae (smoltification). The literature has been summarized by Pickford & Atz (1957), who also reviewed the information available on the effect of thyroxine on metamorphosis in the eel and salmonids. It was found that thyroxine was able to induce changes in salmonids which could be compared to those occurring during smoltification. The process of metamorphosis is usually completed shortly before or after the onset of migration. Therefore, if the increase in thyroid activity would only be associated with this process one would expect this activity to decrease as soon as metamorphosis is completed, thus, shortly after the onset of migration. However, the data given in Table 1 show that this is not the case, but that thyroid activity remains high during the whole migration period. Furthermore, in animals which do not undergo metamorphosis at migration time, e.g. the stickleback, thyroid activity still shows an increase at the onset of migration. Therefore, although metamorphosis may have some additional effect on thyroid activity, it cannot be the only cause of the hyperactivity occurring at the time of migration.

Gonadal maturation

It has been suggested many times that maturation of the gonads (or their regression), which takes place in many migrating species at the onset of migration, may be responsible for the increase in thyroid activity at that time. This point will be discussed in detail later (p. 46), but it can be stated here, that the evidence supporting this view is slight.

Diet and iodine contents of the water

It is known that diet affects thyroid activity (Marine, 1914 ; Hoar, 1951 a, 1959). As some migratory species change their feeding habits at the onset of, or during migration (e.g. the eel and adult salmon) it is possible that this might affect thyroid activity, also because of the consequent change in metabolism. However, as a change in diet occurs only in a few species of migrants, it is not very likely that in general this factor will be the only cause of thyroid hyperactivity.

There is also evidence that the iodine contents of the water may affect thyroid activity (see summary by Pickford & Atz, 1957 ; Hickman, 1959). Thyroid activity tends to be higher in animals living in iodine poor water, than in those living in iodine rich habitats. As fresh water has a much lower iodine content than sea water, this may influence thyroid activity in diadromous fish. However, there is no reason to assume that the increase in thyroid activity at the onset of migration would only be caused by the iodine contents of the water, particularly not if one considers that this increase also takes place in animals beginning their migration in the sea, which is rich in iodine.

The final conclusion from this discussion is that many factors operating at the time of migration may influence thyroid activity, but there is very little evidence that they are the only factors involved. There are more arguments in favour of the view that there is a primary increase in thyroid activity at the onset of migration caused by other factors than the ones discussed above, although the latter may have some additional influence.

(iv) *The way in which thyroid hormone may affect migration*

In the previous discussions many arguments have been given supporting the hypothesis that the increased thyroid activity at the onset of migration is a causal factor inducing migration-disposition. The question now arises how thyroid hormone may exert its influence on migration. As has been mentioned before, migration can be considered to consist of locomotory movements combined with special orientation components. As yet migratory behaviour has hardly been analysed except in the case of juvenile salmon (Hoar, 1958). There are two possible ways in which thyroid hormone may exert its influence, namely either by affecting migratory behaviour directly, or indirectly by means of affecting other processes.

There are some arguments that thyroid hormone may directly affect the locomotory component of migration. Hoar, *et al.* (1952), and Hoar, *et al.* (1955), reported that treatment of goldfish and juvenile salmon with thyroxine caused an increase in general activity. Fontaine (1954, 1956 a) also suggested that thyroid hyperactivity causes an ' agitation motrice ' which may be partly responsible for the onset of migration.

There is also evidence that thyroid hormone may affect migration indirectly. Hoar, *et al.* (1952, 1955), have shown that thyroxine increased the responsiveness of goldfish and juvenile salmon to water currents and electric stimuli. Hoar (1953) mentioned that salmon smolt are more sensitive to external stimuli than parr and suggested that thyroid hormone may have been responsible. Increased sensory perception may be involved in migration by making the animals more sensitive to external ' releasing ' stimuli and/or navigational clues. The above findings are in agreement with the current view that hormones affect the responsiveness of the nervous system to external stimuli, and that a higher level of hormone intensifies the behaviour affected by the hormone (Beach, 1948). There is also evidence that thyroid hormone may affect migration indirectly by affecting metabolism and osmoregulation, both undoubtedly involved in migration. The above-mentioned influence of thyroxine on locomotory activity may not have been a direct effect, but may have stimulated metabolism and in this way general activity. However, the role of thyroid hormone in fish metabolism is still rather obscure (Pickford & Atz, 1957 ; Hickman, 1959). It has been mentioned before that at the onset of migration the increase in thyroid activity is correlated with a change in osmotic balance. Lampreys seem to be the only animals studied so far in which the change in osmoregulation was not associated with thyroid hyperactivity (Fontaine & Leloup, 1950 b). It was also mentioned that there are some arguments indicating that osmotic disorder may enhance thyroid activity. On the other hand, however, there are also indications that thyroid hormone may be the cause of changes in osmoregulation. Koch & Heuts (1942) reported that administration of thyroxine to sticklebacks in sea water disturbed their osmoregulation in that chlorides penetrating from the environment were no longer excreted, but accumulated in the blood and finally caused death. The influence of thyroxine and thyroid hormone on salinity preference and tolerance may also be mediated by induced changes in osmotic balance. Therefore, salinity tolerance and preference have been placed under the heading of ' Osmotic Balance ' in Fig. 1, although a causal relationship has not been proven as yet. The above observations seem to indicate that changes in thyroid activity may induce changes in osmotic balance. Fontaine & Koch (1950), Koch (1955) and Fontaine (1954, 1956 a) assumed that osmotic disorder might be the compelling force driving the animals out of their habitat in search for a new environment to which they will be better adapted. On the basis of these considerations it seems possible that thyroid hormone may affect migration indirectly by causing changes in osmotic balance. Osmotic changes may also cause the changes in orientation towards fresh and salt water found in the salinity tests and, as has been mentioned earlier, this may be one of the orientation components used during migration (p. 42). It is also possible that osmotic changes may affect locomotion, for one could think that animals may continue swimming as long as they are not adjusted to their environment (ortho-kinesis).

Little is known about the way in which thyroid hormone affects osmoregulation. In fishes various organs are involved in this process, namely the skin, gills, alimentary tract and kidneys. It might be possible that thyroid hormone

affects the chloride cells in the gills, for Hoar (1951 a) reported that TSH induced the appearance of these cells in salmon parr. Thyroxine also affects the skin (La Roche, *et al.*, 1950 ; La Roche & Leblond, 1952). These investigators found that administration of thyroxine caused thickening of the epidermis in salmonids, which may be involved in bringing about the increase in salinity tolerance after administration of this substance. The observation that thyroid hormone in one species causes a fresh water preference and decreased salinity tolerance (stickleback), whereas in another species it induces a salt water preference and increased salinity tolerance (juvenile salmon) offers a very interesting problem which at the moment is difficult to solve. Pickford & Atz (1957) have pointed out that euryhalinity has evolved in many different species of fish, and apparently in many different ways. However, in the above case it is difficult to conceive how this difference in response of the osmoregulatory mechanism to thyroid hormone has been achieved. Before an attempt can be made to explain this difference one should first know how thyroid hormone is involved in osmoregulation in different species of fish, migratory as well as non-migratory. Only if this is known a fruitful attempt can be made to explain the difference in response to thyroid hormone. It should be borne in mind, however, that as yet there is no absolute proof that thyroid hormone causes a change in osmoregulation, although it affects salinity tolerance and preference. It remains possible that the correlation between thyroid activity and osmotic changes has to be difierently interpreted, and this would alter our approach to the problem. This point awaits further investigation.

(b) *The gonads*

Spawning migrations to fresh water occur in sticklebacks, Atlantic and Pacific salmon and many others, whereas the eel migrates seaward to spawn. Post-breeding migrations back to the sea take place in the stickleback, Atlantic salmon and many others (Pacific salmon die after spawning). Only in immature migrants, like juvenile sticklebacks and salmon, there is no apparent connection between migration and the gonadal cycle. This general correlation between gonadal cycle and migration has favoured the widespread view that gonadal development or regression may be a causal factor in the induction of migration. However, there is very little evidence supporting this view. Fontaine (1954, 1956 a) has repeatedly emphasized the fact that among juvenile salmon migrating seaward some are found with quiescent or developing gonads, whereas others, which had spawned prematurely with the adults, had regressing gonads. These observations make it difficult to conceive how gonadal processes can be a causal factor in the induction of migration.

There is also experimental evidence indicating that the gonadal cycle may not be a causal factor in the induction of migration. This was found in experiments in which changes in salinity preference were used as criterion for the induction of migration-disposition. Baggerman (1957, 1959) exposed normal and gonadectomised sticklebacks to a constant day length of 16 hours combined with a temperature of 20° C. Under these conditions normal animals show alternative reproductive and non-reproductive periods correlated with periods

of fresh and salt water preference respectively (Fig. 3, see also Fig. 4). As can be seen from Fig. 3 gonadectomised animals show exactly the same alternation in periods of fresh and salt water preference as the normal fish. Therefore,

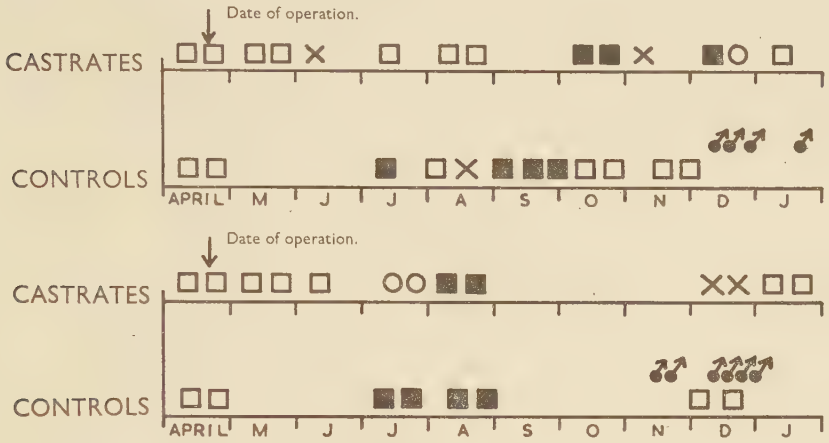

Fig. 3.—Changes in salinity preference occurring in normal and gonadectomised sticklebacks kept under external conditions inducing gonadal maturation in normal fish. The symbol ♂ indicate dates of maturation. For explanation of other symbols see Fig. 2.

the gonads are not an essential factor in the induction of changes in salinity preference. However, they seem to have some modifying effect on the time at which this induction takes place, for Fig. 3 shows that the onset of the changes in salinity preference in the gonadectomised animals lagged behind that in normal fish. An explanation for this phenomenon will be given on page 50. In addition to the above findings it was shown that in castrated fish thiourea was able to induce a salt water preference, just as in normal fish. The conclusion is that the gonadal cycle is not a causal factor in the induction of changes in salinity preference and, therefore, probably also not in the induction of migration-disposition. However, gonadal hormones, although perhaps not indispensable, may still influence migration by exerting some influence on certain processes and/or behaviour patterns connected with migration. Hoar, *et al.* (1952, 1955) reported that gonadal hormones increased metabolism and the speed of swimming in fish, in addition to making them more sensitive to external stimuli such as water currents and electric shocks. This may indicate that gonadal hormones may either affect locomotion directly, or by way of stimulating metabolism. They also seem to act upon sensory perception, which at the time of migration may make the animals more sensitive to external stimuli ' releasing ' migration and/or to navigational clues.

(c) *The interrenal tissue*

There is some evidence that the activity of the interrenal tissue (secreting cortical hormones) undergoes a change at the time of migration. Fontaine & Hatey (1950, 1954) found that the concentration of gluconeogenic steroids

was much higher in salmon smolt than in parr. They also reported that adult salmon on their spawning migration had high titres of cortical steroids in their blood. These observations indicate that cortical hormones may in some way be involved in migration. There is good evidence that in fishes, as in higher vertebrates, interrenal hormones are involved in regulating metabolism and the hydromineral balance (see reviews by Pickford & Atz, 1957 ; Fontaine, 1956 b ; Chester Jones, 1956 ; Chester Jones, *et al.*, 1959, and his article in this symposium). It is possible, therefore, that cortical hormones may affect migration by influencing these two processes. However, so far only the influence on osmoregulation has been studied in migrating fishes and the available information on this point is rather contradictory. In the silver eel the loss of chlorides at migration time was not influenced by treatment with mammalian cortical steroids or with DOCA (Callamand, 1943). This might indicate that in the migrating silver eel the interrenal tissue is not involved in osmoregulation. On the other hand Fontaine & Motais (1956) reported that injections of DOCG (desoxycorticosterone glucoside) were able to abolish the strong halophobic reactions in the elver and to prolong their survival time in sea water. These findings seem to indicate that cortical hormones might be involved in elver migration. In this case one has to assume that in elvers migrating to fresh water the activity of the interrenal tissue decreases, causing the typical strong halophobic reactions. This would be in line with the observations that in seaward migrating salmon interrenal activity seems to be increased (see above). The conclusion is that cortical steroids may be involved in migration by influencing osmoregulatory processes. The information is not sufficient to conclude whether interrenal activity is a causal factor in the induction of migration, or whether it is a secondary effect.

(d) *The pituitary*

There is indirect evidence that the activity of the pituitary gland is a causal factor in migration. In the first place cytological changes have been found in the gland at the time of migration (Fontaine & Olivereau, 1949). In the second place, since it has been shown that various endocrine glands may play a role in the causation of migration, it follows that pituitary activity must also be a causal factor because of its regulating influence on the activity of these glands. However, information coming from experiments designed to show the role of the hypophysis in migration in a more direct way is scarce and often contradictory.

As has been shown there is strong evidence that thyroid activity is a causal factor in migration. In fishes, as in higher vertebrates, the thyroid gland is under pituitary control. However, experiments in which TSH was used have revealed contradictory results with regard to its influence on migration. Hoar (1951 a) reported that TSH induced the appearance of chloride cells in the gills of salmon parr. Smith (1956) found that TSH failed to promote salinity tolerance in *Salmo trutta*, although thyroxine was effective. Gineste (1955) has reported that TSH increased general activity in the eel. These differences in response may be partly or wholly due to species specific differences in TSH.

It has been mentioned that the gonads are not a causal factor in the induction of changes in salinity preference. However, in the experiments with gonadectomised animals (p. 46) one could imagine that gonadotropic hormones may have played a role, for they continue to be secreted after gonadectomy. Preliminary experiments on this subject failed to show an effect of (mammalian) gonadotropins on salinity preference (Baggerman, 1957), although the experiments should be repeated before a definite conclusion can be drawn. Since there are indications that gonadal hormones may affect metabolism, locomotion and sensory perception it is clear that the pituitary must be involved too by means of its secretion of gonadotropins (GTH).

There is some evidence that in fishes, as in other vertebrates, the secretory activity of the interrenal tissue is under pituitary control. Since cortical steroids may be involved in osmoregulation during migration, the pituitary must also play a role by means of its secretion of corticotropic hormone (CTH). However, Smith (1956) reported that (mammalian) ACTH failed to promote salinity tolerance in *Salmo trutta*.

Apart from glandotropic hormones the pituitary also secretes a number of other hormones which might play a role in migration. Smith (1956) found that anterior lobe powder promoted salinity tolerance in *S. trutta*, and that growth hormone (STH) had an even more striking effect. In this connection it should be remembered that during smoltification juvenile salmonids show an accelerated growth rate and at the same time develop an increase in salinity tolerance. The possible connection between these phenomena and the ways in which growth hormone may affect salinity preference are discussed in Smith's article.

In fishes the neurohypophysis secretes hormones that are concerned in water metabolism, as it does in higher vertebrates (Fontaine, 1956 ; Pickford & Atz, 1957). However, Smith (1956) reported that fish posterior lobe preparations failed to promote salinity tolerance in *S. trutta*. This would mean that the neurohypophysis may not be involved in processes connected with salinity tolerance, and possibly not in migration. However, since there is no more information available on this point further investigation must be awaited before a conclusion can be drawn.

Although in the previous discussion some positive findings were reported on the influence of pituitary hormones on osmoregulatory processes the curious fact remains that so many investigators found that hypophysectomy did not impair the viability of the animals to the medium to which they were adapted prior to the operation. Pickford & Atz (1957) have summarized the information. In migrating species of fish information is still scarce and apparently contradictory. In the yellow eel hypophysectomy had no effect on the ability of the animals to survive in fresh water, or to withstand abrupt transfers from fresh to salt water and *vice versa* (Fontaine, *et al.*, 1949 ; Callamand, *et al.*, 1951). However, in the elver removal of the pituitary tended to diminish the state of halophobia in migrants (Vilter, 1956), but according to Fontaine (1956 b) this observation should be regarded with some caution.

The general conclusion regarding the role of the pituitary gland in the induction of migration is that there is indirect evidence that the gland must

be involved as a causal factor, because it regulates the activity of other glands playing a role in the initiation of migration. Furthermore, it may also influence certain processes connected with migration by secretion of non-glandotropic hormones. More indirect evidence for the role of the pituitary in migration will be given later (p. 52).

(e) *Mutual relationships between endocrine glands*

It is very likely that the induction of migration-disposition does not depend on changes in activity in one endocrine system only, but on more processes intricately working together. This assumption seems also inevitable from the point of view that mutual relationships exist between the various endocrine glands. A review of these interrelations has been given by Pickford & Atz (1957), Hoar (1951a, 1957) and only a few of them will be briefly discussed here with respect to their possible role in the causation of migration.

There are some arguments that a mutual relationship exists between the activity of the thyroid gland and the gonads, although its exact nature is still rather obscure. This relationship might be responsible for the observations that both thyroxine and gonadal hormones influence general activity and sensory perception in fish (Hoar, *et al.*, 1952, 1955). In this case administration of one hormone may have affected the secretion of the other, and only experiments with hypophysectomised animals may clarify this point. On page 47 it was shown that the time of onset of changes in salinity preference in gonadectomised fish lagged behind that in normal animals. This might be explained by assuming that gonadal hormones affected the secretion of thyroid hormone (Baggerman, 1957). Thus, gonadal hormones would indirectly help to induce the proper timing of migration.

It is known that a relation exists between the effects of thyroid hormone and growth hormone. This may throw some light on the observations of Smith (1956) that high doses of thyroxine had the same effect on salinity tolerance in *S. trutta* as a low dose of growth hormone. It is possible that in this case one of the hormones stimulated the secretion of the other, or that their effects were synergistically augmented.

(2) *Factors influencing the endocrine systems involved in migration*

In the previous sections it has been discussed how changes in activity of various endocrine glands may be involved in migration, and the next question that arises is how these changes were brought about. In the first place one could think that cyclical changes in external conditions might be responsible, and in the second place that endogenous rhythmic processes might play a role. These two possibilities will be discussed below.

(a) *External ' priming ' factors*

These factors are thought to prepare the animals for future action in that they make them physiologically ready to respond to external ' releasing ' factors as soon as these occur. Thus, ' priming ' factors are thought to have a long-term effect. In contrast with this long-range effect ' releasing ' factors are thought to exert their influence within a relatively short period of time

(short-term effect). However, as the exact nature of releasing and priming factors is unknown, and since different investigators tend to attach different meanings to them, both terms are placed in quotes here. They are only used because at the present state of our knowledge it still seems useful to distinguish between them in the above described way, although there are cases where their actions seem to merge.

(*i*) *The influence of day length*

Most migrations occur at a time of the year when day length is either increasing or decreasing, and it is *a priori* not unlikely that this factor may be involved in inducing changes in the endocrine systems. It has been shown that the length of the daily photoperiod controls the induction of the physiological condition associated with migration. This has been reported in sticklebacks and juvenile salmon (Baggerman, 1957, 1959, 1960). Only the main results will be given here. In sticklebacks (at the beginning of gonadal development) that have a preference for salt water a change in preference can be induced by exposing them to a long day length (16 hours) and a high temperature (20° C.). In such animals a short day length (8 hours) at the same temperature will prevent this change, even when the fish are kept under this condition for over one year. Furthermore, cyclical changes in salinity preference can be induced in animals maintained for long periods of time under a constant long day length and high temperature (Fig. 4, see also Fig. 3).

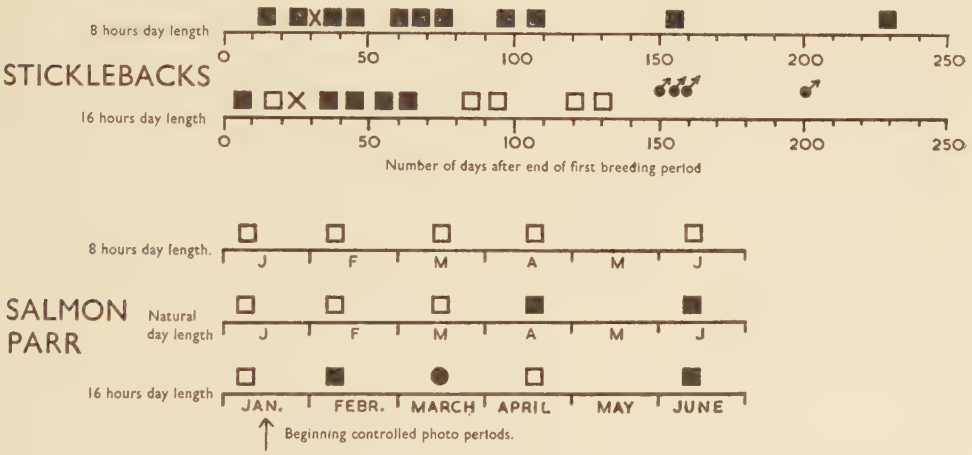

Fig. 4.—The effect of the length of the daily photoperiod on salinity preference in sticklebacks and coho salmon parr kept under constant artificial daily photoperiods and constant temperatures. For explanation of symbols see Fig. 2.

However, such cyclical changes do not occur in animals kept for the same period of time under a constant short day length and high temperature and these animals maintain their initial salt water preference. The conclusion is that the induction of a change in preference depends on the length of the daily photoperiod. The fact that a long day length induces a fresh water preference ties in with the observation that sticklebacks migrate to fresh water in spring when the days are lengthening.

Similar experiments were carried out with juvenile Pacific salmon. Fig. 4 shows that a short day length has an inhibiting effect on the change in preference from fresh to salt water, whereas a long day length induces an earlier change than under natural conditions, or under a short day length. The temperature was the same in all groups (6–10° C.). The conclusion is that day length controls the induction of changes in salinity preference in juvenile salmon. This is in agreement with the observations that in spring, when the days are lengthening, young salmon migrate seaward.

The conclusion from these two series of experiments is that in sticklebacks as well as in juvenile salmon the length of the daily photoperiod controls the time at which changes in salinity preference take place, and which probably reflect induction of migration-disposition. It is interesting to note that in sticklebacks a long day length induces a fresh water preference and migration to fresh water, whereas in young salmon it causes a salt water preference and seaward migration. This difference is most probably caused by a different adaptation to this external condition. In addition, the fact that the migrating stickleback in spring is a maturing animal, whilst the young salmon is immature may also play a role. Different adaptations to the same external condition are not rare in nature. Another example is the difference in response to day length between vertebrate species which breed in spring and those breeding in autumn.

(ii) *The influence of temperature*

Temperature is another factor which shows annual cyclical changes that are correlated to some extent with the onset of migration. There is some evidence that temperature plays a role in the induction of migration. When the early months of the year are mild sticklebacks migrate to fresh water earlier than when it is cold at this time of the year (Baggerman, 1957). In young Pacific salmon the onset of seaward migration occurs earlier when the weather in spring is relatively mild (Foerster, 1957). This author reports that a slight rise in temperature may cause an exodus of sockeye smolts from lakes, and that in cold years the reaching of a threshold temperature of 4·4° C. has the same effect. In the first case it is not clear whether the slight rise in temperature acted over a relatively short period of time (' releasing ' factor), or over a longer period (' priming ' factor). In the second observation temperature may have been a ' priming ' factor, because the reaching of a threshold temperature apparently took several weeks. It is interesting again to note that an increase in temperature causes seaward migration in young salmon and migration to fresh water in sticklebacks.

(iii) *Endocrine systems affected by day length and temperature*

It is very likely that the effect of day length on migration is mediated by the hypothalamus-pituitary axis stimulating the pituitary to activate various other glands. The most important system involved in migration that seems to be activated is the pituitary-thyroid mechanism, for this gland is hyperactive in the majority of diadromous fishes at the time of migration. However, there is hardly any direct experimental evidence that the photoperiod affects

this system, and this point awaits further investigation. There is a considerable body of evidence that the length of the daily photoperiod stimulates the pituitary-gonad system (see reviews by Baggerman, 1957 ; Hoar, 1957 ; Pickford & Atz, 1957). There is no information concerning the possible influence of day length on the secretion of other glandotropic and of non-glandotropic hormones by the pituitary.

More information is available on the effect of temperature on endocrine mechanisms, although most of it is highly contradictory (Pickford & Atz, 1957). The effect of temperature on salinity preference, which will be described later (p. 54) is probably mediated by stimulation of the pituitary-thyroid system. Olivereau (1955), working with *Salmo gairdnerii* and *Anguilla anguilla*, showed that at low temperatures there was hardly any evidence of stimulation of the thyroid, whereas at 20° C. there were the usual histological signs of activation. Fontaine, *et al.* (1955), reported that in *S. gairdnerii* administration of TSH at low temperatures (8° C.) had hardly any effect on the thyroid, whereas it did have effect at higher temperatures (20° C.). These findings seem to indicate that temperature may affect thyroid activity by means of stimulating the pituitary to produce more TSH and/or by making the thyroid more sensitive to TSH. However, there are other authors who reported no effect of temperature on thyroid activity, or even opposite effects. As these observations mainly concern non-migratory species of fish it would lead too far to discuss them here. Pickford & Atz (1957) have summarized the data.

It is known that temperature affects the gonadal cycle (see reviews cited above in connection with day length), but no information seems to be available on the influence of temperature on secretory activity in other glands involved in migration.

(b) *Endogenous rhythmic processes*

There is some evidence that the endocrine mechanism is influenced not only by external factors, but also by endogenous rhythmic processes. An internal rhythmic process is defined here as an activity that occurs rhythmically when external conditions are kept constant. The duration of this internal cycle is usually different from that occurring under natural conditions. Further, the external conditions should be such that they do not inhibit the manifestation of the endogenous cycle. Theoretically rhythmic processes may either induce an increase in endocrine activity, or a decrease. In the present section information will be discussed concerning an internal rhythmic process which causes a decrease in activity of endocrine systems involved in migration. Full information on the experimental data can be found in Baggerman (1957), and only the main points will be discussed here.

Sticklebacks exposed to constant external conditions at the beginning of the reproductive period continue to breed for a certain period of time after which breeding is discontinued. This happens under a constant long day length (16 hours), as well as under a constant short day length (8 hours), in both cases at a constant temperature of 20° C. Correlated with the termination of breeding is a change in preference from fresh to salt water. This

indicates that an internal process must have been responsible for both the termination of breeding and the change in salinity preference. It is very likely that this factor is some kind of pituitary exhaustion affecting both the gonads and the thyroid gland. Furthermore, under a constant long day length and high temperature alternating periods of breeding and non-breeding occur, correlated with alternating periods of fresh and salt water preference (Figs. 3 and 4). The duration of this cycle is 200 days, thus, shorter than under natural conditions where it lasts one year. The alternation under the constant external conditions is most likely caused by a rhythmic pituitary exhaustion and its subsequent re-activation by the long day length with, possibly, a refractory period in between. The conclusion, therefore, is that an endogenous rhythmic process is involved in the induction of a change in preference from fesh to salt water and, thus, possibly in induction of seaward migration-disposition in sticklebacks. A similar phenomenon may occur in other species of fish in which post-spawning migrations take place (e.g. Atlantic salmon).

IV. EXTERNAL 'RELEASING' FACTORS INVOLVED IN MIGRATION

As has been mentioned in § I a co-operation between two sets of factors is required for the induction of migration. One is the proper physiological condition and the other appropriate external 'releasing' factors. The nature of the physiological condition and the factors responsible for its induction have been dealt with in the previous chapter and we may now discuss the external 'releasing' factors. As was mentioned on page 50 'releasing' factors are thought to have a short-term effect, i.e. they evoke a response within a relatively short period of time, something in the order of a few hours to a few days.

(1) *Temperature*

Field observations seem to indicate that in sticklebacks and juvenile salmon, a sudden rise in temperature may cause the onset of mass migrations, but no reliable data are available on this point. In sticklebacks it was shown that between December and February a sudden rise in temperature (e.g. from 4–10° C. within 24 hours) caused a change in salinity preference from salt to fresh water within one or two days (Baggerman, 1957). It seems, therefore, that in this case the abrupt change in temperature 'released' the change in preference. In nature it may have caused the onset of migration to fresh water. This interpretation is supported by the fact that a similar sudden rise in temperature in November did not induce a change in the salt water preference of the animals. This seems to indicate that at that time of the year the animals were not yet in the proper physiological condition (migration-disposition) to be able to respond to the 'releasing' effect of the sudden rise in temperature.

Keenleyside & Hoar (1954) found that in experimental situations a sharp rise in temperature induced a negative rheotaxis in chum and coho fry initially showing a positive rheotactic response ; sockeye fry, however, did not change their positive rheotaxis. In chum fry, which had remained in fresh water past the time of peak seaward migration of the species, a rise in water tempera-

ture seemed to induce or enhance active swimming with the current, which would tie in with the above finding (Hoar, 1958). However, the significance of the change in response in coho fry is obscure, as this species usually does not migrate to the ocean as fry. The same holds for the absence of a response in sockeye fry as these fish occasionally migrate seaward as fry.

(2) *Light intensity*

Hoar (1953, 1958, 1959) found that fry of schooling species of Pacific salmon (chum, pink and sockeye) become active when the light intensity drops at night and swim near the surface of the water. In this way their positive rheotactic response, which they show in the day time, fails because of the absence of visual clues and the animals are displaced downstream towards the ocean. This does not necessarily mean that migration of these species to the sea is only a passive displacement, for it was observed in addition that some of the animals moved faster than the current and, therefore, swam actively downstream. If the fish do not reach deep water before the light intensity increases the next morning they hide under stones on the bottom until the light intensity drops again in the evening. In this way fry migrate seaward in a series of nocturnal leaps induced by the falling light intensity. Species that do not migrate seaward as fry, such as *Oncorhynchus kisutch* W., *Salmo salar* and *S. gairdnerii*, show a completely different response with regard to light intensity as the other three species. They are territorial in the day time and become inactive when the light intensity drops. In this way they are not displaced seaward as fry.

(3) *River discharge*

Several investigators are of the opinion that river discharge may control the onset of migration in salmon. Thus Davidson, *et al.* (1943) and Huntsman (1945) believed that river discharge may control the upstream movement of sexually mature salmon. Fontaine (1956 a) stated that when the water level in a river rises abruptly it very frequently results in a mass exodus of seaward migrating juvenile Atlantic salmon. Hoar (1951 b) mentioned that a sudden rise of the water level induced an increase in the rate of downstream displacement in juvenile Pacific salmon.

(4) *Meteorological factors*

Fontaine (1956 a) mentioned that a drop in barometric pressure may result in an abrupt onset of seaward migration in smolts. However, as such a drop in barometric pressure is frequently correlated with rainfall and subsequent river discharge, it is difficult to ascertain which of these factors might be responsible.

The above discussion shows that conclusive data on the nature of external ' releasing ' factors are extremely scarce. Furthermore, it is also unknown how they may exert their influence. It is possible that they affect migratory behaviour directly, or that they act on one or more processes in the body which in their turn induce migration. In the latter case it might be possible that the external stimuli act upon the same processes as described to play a

role in the induction of migration-disposition. If so, the ' releasers ' would exert their influence at the very last phase of this induction. An argument in favour of this view would be the observation that between December and February a sudden rise in temperature induces a change in salinity preference, which is an essential component of migration-disposition, while at the same time it may release mass migrations to fresh water (p. 54). However, it is also possible that the ' releasers ' affect other processes than the ones mentioned above. These two possibilities have been indicated in Fig. 1. The solid lines connecting the ' External Releasing Factors ' with the ' Physiological Condition ', and the latter with ' Migration ' express that the former two components have to act together to bring about the onset of migration. The dotted line indicates that it is not known which physiological components are affected by the ' releasers '.

V. SUMMARY

On the basis of available information it is concluded that before migration occurs a change takes place in the physiological condition of the animals rendering them susceptible to appropriate external factors ' releasing ' migration. The changes in physiological condition comprise changes in secretory activity of endocrine systems, of which the pituitary, thyroid, gonads and interrenal tissue seem to be the most important glands involved. The hormones may directly affect migratory behaviour, or induce changes in physiological components such as osmoregulation, metabolism and sensory perception, which in their turn may affect migration. The changes in endocrine activity are controlled by external ' priming ' factors such as the length of the daily photoperiod and temperature, and by endogenous rhythms. In animals in which the proper physiological condition has been induced various external stimuli like temperature, light intensity, river discharge and meteorological factors are able to ' release ' migration.

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GENERAL DISCUSSION OF PAPER

Chairman : Professor Sir Solly Zuckerman, C.B., F.R.S.

Mrs Woodhead : I have been studying the seasonal changes in the activity of the thyroid gland of the Barents Sea cod in relation to its seasonal migrations, and the results show some agreement with those of Dr Baggerman. The adult cod carry out an annual spawning migration often of more than 800 miles from the feeding areas in the Barents Sea to the spawning grounds inside the Lofoten Islands on the north Norwegian coast ; the cod does not encounter a change in salinity during this spawning migration. The immature fish also show seasonal movements, migrating southwards in the winter and, as they become older, the length of this southward migration increases, until in some of the oldest immature cod the migration extends almost to the spawning grounds of the adults ; this migration, which does not end in spawning, has been called a ' dummy run '. I found that the thyroid of the mature fish became active as the fish began their spawning migration and activity continued until spawning time ; in spent fish the gland had re-entered the resting condition. In the mature cod the thyroid was active throughout the period of gonadal maturation but a similar cycle of thyroid activity also occurred in the immature cod, coinciding with their winter migration. Furthermore the duration of thyroid activity in the immatures increased as the length of the ' dummy run ' increased. The gonads of the immature fish remained inactive and it therefore appeared that the greater length of the migration of the older, immature cod was related to the increased duration of thyroidal activity.

Dr Baggerman : The study of the endocrine control of migration in diadromous species of fish is complicated by the change in osmotic medium requiring special endocrine regulations. It is, therefore, very encouraging to hear that the change in thyroid activity found at the onset of migration in diadromous species also occurs in an oceanodromous species which does not encounter a change in osmotic medium. This observation supports the view that the thyroid gland plays an important role in the causation of migrations either or not comprising changes in osmotic medium.

The fact that immature cod also carry out migrations favours the view that the gonadal cycle plays a less important role in the initiation of migration than seems to be generally accepted, and ties in with the findings mentioned in the present paper. The increase in thyroid activity which was also found in these immature migrants once again stresses the important role of the thyroid gland in migration.

P. M. J. Woodhead : Why does Dr Baggerman believe that the increased thyroid activity observed one month before changes in salinity preference are good evidence for the thyroid controlling these changes ? From her experimental results would she not expect the change in preference more or less to coincide with increased activity of the thyroid ?

Dr Baggerman : The data given in Table 1 show that the increase in thyroid activity occurs before the onset of migration, but they give no informa-

tion on the length of the period elapsing between the occurrence of the two phenomena. The onset of migration took place some time between the middle of April and the beginning of May, but no samples of fish were examined during this period. I would not expect *a priori* that the change in salinity preference would more or less coincide with the increase in thyroid activity, as it is known that quite often it may take considerable time (up to several weeks) before a behaviour pattern changes as a consequence of a change in endocrine activity.

THE LIFE-CYCLE OF THE ATLANTIC EEL

BY

UMBERTO D'ANCONA

University of Padua, Italy

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INTRODUCTION

Among the families which have colonized littoral and inland waters, the Anguillidae is of particular interest. This family, though related to other marine groups, comprises solely the genus *Anguilla*. This genus according to the most recent taxonomical revisions, includes sixteen species, the majority of which are Indo-Pacific (Ege, 1939). Only two species are represented in the Atlantic area, namely *A. anguilla* (L.) and *A. rostrata* (Le Sueur). My talk will be restricted to these two species, with special reference to the former both because it has been the subject of my own researches and because it has been the more thoroughly investigated.

The adults of these two species occupy clearly separated distribution areas. On the one hand, *A. anguilla* is found along the east coasts of the North Atlantic, from the coasts of the White Sea, western Europe, the Mediterranean, the Black Sea and West Africa so far south as *ca.* 25° lat. N ; its distribution also embraces Iceland, the Faeroes, Azores, Madeira and the Canary Islands (Ege). On the other hand, *A. rostrata* is distributed along the west coast of North America from Labrador to the southern part of Mexico, Panama, the Guianas, West Indies, the Bermudas and the southern part of the west coast of Greenland.

The differences between the two species are small. The chief distinctive character consists in a difference in the number of vertebrae. In *A. anguilla* the number of vertebrae ranges between 110 and 119 (average 114·73) of which 44 to 47 are preanal, whereas in *A. rostrata* there is a range of between 103 and 111 (average 107·23) of which 41 to 45 are preanal. There is also a difference in the average maximum value of distance between verticals through the anus and the origin of the dorsal fin, which according to Ege is *ca.* 11·2 per cent of the total length in *A. anguilla* and in *A. rostrata*, *ca.* 9·1 per cent. Within the two species a great phenotypical variability is to be found ; this is

especially true for the European eel, for which Kaup (1856) listed as many as fifteen different species. Such considerable variety of phenotypes can be related to the great variety of environments in which European eels live. The species show an extremely wide ecological valency, for they are able to stand a wide range of temperature, salinity, feeding and light conditions. The most conspicuous peculiarities of this species are to be found in its biology, for each stage of the life-cycle occurs in a different area and environment. The animal world hardly offers a comparable situation.

However, in spite of the wide geographical distribution, the variety of phenotypes and of environments exploited, there are no correspondent genotypic differences. Researches made so far do not show any evidence of genetic polymorphism. Ege points out that even specimens coming from the most distant distribution areas do not show any significant difference in number of vertebrae or body proportions. It can, therefore, be assumed that the two Atlantic eels represent two clearly distinct monotypic species. Such an assumption looks, however, quite surprising if one considers, especially in the European eel, the wide distribution and the variety of environment in which the better known stages of its life-cycle occur.

SPAWNING AREA AND LARVAL LIFE

The genotypical uniformity of the European eel can be related to the fact that only one spawning area is known so far. In the past, some students of the problem, mainly Italian and among them the writer (D'Ancona, 1936), have questioned the assumption that all European eels, especially the Mediterranean ones, make such a long journey in order to reproduce in the Sargasso Sea (22–30° lat. N ; 48–65° long. W). However, reliable evidence for a special spawning area has not yet been found, nor is there a clear demonstration that the Mediterranean eels migrate to the Atlantic (Ekman, 1932).

Although there are some data which do not support Schmidt's (1912) theory, nevertheless, it is based upon the logical interpretation of a rich collection of facts. It is difficult to see why small leptocephali (60 mm) are found in the Mediterranean Sea much more eastwards than in the Atlantic. It is also difficult to understand the reason why eels so abundant in the Mediterranean must necessarily go through Gibraltar—where they have never been captured—searching westwards for a spawning area whose environmental conditions are not much different from those of the Tyrrhenian and Ionian Seas. Nevertheless, so far a second spawning ground of the European eel has not been discovered. Although investigations in the Mediterranean area have not been carried out so intensively as in the Atlantic, yet we must admit that according to our present knowledge of the problem the only certain spawning area is the one pointed out by Schmidt.

In order to throw some light on the still dubious aspects of the question, Tucker (1959 a, b) has recently worked out a new interpretation, certainly ingenious, though in my opinion not sufficiently documented. According to Tucker's hypothesis, European and American eels belong to the same species, deriving from a common spawning area. From there, due to the action of currents, some are carried toward the European continental waters, and some

toward American waters. The former would never return to the departure point and would, therefore, be maintained only by the latter. A similar view was already proposed by Ekman (1932) for the Mediterranean eels, but was discarded by the same author.

Tucker's hypothesis is based upon a double line of argument. One concerns the morpho-physiological condition of silver eels when they have reached the sea. In his opinion these eels show degenerative changes and appear close to death ; to this problem I shall return later. Tucker's other argument is based on the hydrographic conditions of the Atlantic and on the possible influence exerted by different temperatures, on the formation—during embryonic life—of different numbers of somites, and consequently of vertebrae.

Whereas Schmidt believes that European and American eels have two distinct spawning areas, though close to each other and perhaps partly overlapping, Tucker considers that there is one single area. In this area of the Atlantic, situated between 20° and 30° lat. N, the eggs laid at a depth of 400–700 m would find a uniform temperature until they reach the 18° C isotherm at the depth of 300 m. In the southern part of this area eggs and larvae which are moving upward, find in the last 250 m a temperature reaching 25° C, with a difference of 4° C between the 200 and 100 m levels. In the northern part, eggs and larvae do not undergo such a shock effect since the temperature does not exceed 20° C. According to the effect of temperature on the number of myomeres observed by Schmidt (1920) and Tåning (1952) during embryonic development in trout, Tucker claims that in the larvae of the northern area the number of myomeres is increased to 110 to 119, whereas in those of the southern area it is stabilized around 103 to 111. Moreover, the larvae of *A. rostrata* would be carried westward by the Antilles current when they are still at a great depth whilst the larvae of *A. anguilla* would move horizontally more slowly and only when they approach the surface. The former, occupying a warmer environment richer in plankton, would grow faster, probably with a shorter larval life.

On the basis of these premises, Tucker thinks that the differences between European and American eels are not genetically controlled, but due only to environmental influences. According to this interpretation, the pre-larvae of the two species should reach waters with different temperatures at lengths less than 6 mm when according to Schmidt's (1932) results the number of myomeres is already peculiar to the species. That the temperature differences mentioned above may induce such pronounced differences of development is, in my opinion, a somewhat arbitrary extension of the experimental results obtained by Schmidt and Tåning. However, the fundamental objection to Tucker's hypothesis lies in the fact that the larvae develop in two clearly distinct groups without intermediate stages. Environmental conditions, as pointed out by Tucker, do not seem so distinct in the two parts of the spawning area to such a degree as to explain the clear-cut separation of the variability curves of vertebral numbers in the European and American eels (D'Ancona, 1959).

Although Schmidt's theory still has unproven aspects and although the influence of currents, near Gibraltar, in the Kattegat and in the North Sea,

is not sufficient to explain eel migrations, nevertheless, its foundations are more reliable than those of Tucker's theory, which is based on too many unknown factors. Moreover, it is difficult to believe that all European eels represent a dead branch of the species. It is indeed odd that natural selection has not already caused their extinction.

METAMORPHOSIS OF LEPTOCEPHALI

The distribution of the leptocephali of the European eel in the Atlantic follows a regular pattern, with a progressive increase in size from the west eastwards. The current belief is that their metamorphosis into elvers occurs at the edge of the continental shelf. According to Schmidt, their migration eastwards probably lasts two and a half years. The distribution of the leptocephali in the Mediterranean, whence they can reach the Tyrrhenian and Ionian Seas is not in keeping with this interpretation. It was here that they were first collected and here that Grassi & Calandruccio discovered their metamorphosis into elvers in 1896. This metamorphosis is characterized by a decrease in length and a change from a flattened to a cylindrical shape. There is water loss and a consequent increase in mineralization (Fontaine & Callamand, 1941) and strengthening of the skeleton. During this stage there are changes in the endocrine system with an increased activity of the thyroid and the hypophisis (Murr & Sklower, 1928 ; von Hagen, 1936).

After metamorphosis, the elvers reach the littoral regions and some proceed to inland waters. This migration occurs at different times depending on the area. The rivers of western Europe (Iberian peninsula, France, Ireland and England) are particularly remarkable for the massive runs of elvers which have repeatedly been observed and described. Also in the rivers of the west coast of Italy (Tuscany, Latium) the run is quite abundant. On the outskirts of the distribution area of eels, the runs are smaller since the entrance is made by small groups of individuals which are usually no longer transparent elvers of the younger stages but already partly pigmented and sometimes showing the commencement of growth. This can be seen in the Baltic, in the Adriatic and, to a lesser extent, in the Black Sea (D'Ancona, 1958).

GROWTH

The next stage in the life-cycle is that of the yellow eel. Yellow eels develop in freshwater, either at a considerable distance from the sea, or in littoral waters. This is the trophic stage, during which eels undergo growth and sex differentiation. Both processes occur slowly and in various ways.

The growth of eels shows great individual variability and is strongly influenced by environmental conditions, especially by temperature and the availability of food and space. However, even in the same environment, remarkable individual differences in growth can be observed. Elvers of the same age, reared in the same tank or in the same aquarium, especially if the space is limited, after a few months show differences of size which later become more marked. They also show different voracities to such a degree that cannabalism can occur among the largest and smallest specimens (D'Ancona,

1957 c). This diversity probably originates from differences in the endocrine system, and perhaps also from gradual differences in sex orientation.

There is a relationship between growth and sexual dimorphism, but since sex differentiation occurs—as we shall see later—slowly and gradually, growth dimorphism also takes place gradually at variable rates and becomes more evident only at a more advanced age.

On the whole, females grow faster and are bigger than males even when the males have a longer period of growth. Growth stops with the transformation of yellow eels into silver eels. Therefore, the sizes reached at the silver eel stage by males and females are definitive.

The males of the European eel in the Mediterranean seldom exceed the length of 45–46 cm (D'Ancona, 1937) although Gandolfi-Hornyold (1936) occasionally found specimens measuring up to 49–51 cm. The smallest silver males so far recorded measured 24 cm (Sardinia). Silver females usually exceed 45 cm, though Gandolfi records one silver female from the Lake of Tunisi measuring only 37 cm. The maximum lengths reached exceed 1 m. An exceptional specimen recorded by Gandolfi measured 1·52 m and weighed 2·85 Kg.

It is difficult to determine how long eels take to reach their final size. It is always unsafe to determine age by the examination of scales and otholiths. In continental waters, scales are usually formed after some years of life. Ehrenbaum & Marukawa (1913) and Frost (1945) observe that such a delay is variable. According to Frost it depends more on the length of the eel than on its age. With regard to the eels of the Lagoon of Venice, I find, as a rule, that scales appear during the third year of continental life.

The growth of European eels has been studied by many authors in many countries : Germany (Ehrenbaum & Marukawa, 1913 ; Marcus, 1919 ; Wundsch, 1916) ; The Netherlands (Tesch, 1928) ; Great Britain and Ireland (Jespersen, 1926 ; Frost, 1945, 1950) ; Mediterranean countries (Gandolfi-Hornyold, 1936) and others. The results of these investigations are not easily compared with one another because of the variation in growth rate of the eels studied and the remarkable differences in environmental influence already mentioned.

In Lake Windermere, according to Frost (1945), female eels take from 9 to 19 (average 12·27) years before changing into silver eels and males take from 7 to 12 years (average 9). Further, she notes that in the River Bann, Northern Ireland, males live in freshwater from 6 to 8 years and females 10 to 12 years (Frost, 1950). According to Tesch (1928) in the waters of The Netherlands, males migrate to the sea at the end of the seventh or eighth summer. Gandolfi-Hornyold, who has made many observations mostly on single specimens from various origins, thinks that males become ' silver ' between the eighth and fourteenth year of life and females between the tenth and the eighteenth.

My own observations on the eels of the Lagoon of Venice lead me to believe that males leave continental waters between the fifth and the seventh year and females from the beginning of the seventh or eighth year onwards.

As stated above, these data show great variability, depending in part at least upon environmental factors. It is reasonable to suppose that in the

warm Mediterranean, growth occurs more rapidly than in the colder waters of Northern Europe. But final conclusions cannot be reached as yet because of the difficulty of determining the age of the eels and also because the results of the various authors do not lend themselves to easy comparison due to the differences of method and criteria employed. It must be emphasized that, compared to other fish, eel-growth is influenced more by environmental factors, and that sex differentiation depends more on the size than on the age of the animal. Thus the length of the growing period is strongly influenced by the environment. A further difficulty lies in the fact that an eel when in the silver stage although prevented from reaching the sea, may survive for a long time in freshwater. In this regard Gandolfi-Hornyold records specimens which had lived for 50 to 55 years in fresh water.

SEX DIFFERENTIATION

Sex differentiation is closely related to growth. It is preceded by a comparatively long period in which the gonads appear to be undifferentiated. The fact that the gonads of the eel do not reach full maturity in the environments under our control has hindered investigations for a long time.

While the female gonad of the eel was discovered by Mondini in Bologna (1777), that of the male was discovered only in the year 1874 by Syrski in Trieste. But even with the latter discovery, the problem of sex differentiation has not been solved completely because undifferentiated gonads of young specimens can be mistaken microscopically for male gonads. Moreover, up to a certain stage even histological observation cannot give a decisive answer.

The gonad of the young eel does not have a recognizable sex difference and it is not possible to make a cytological distinction between ovo- and spermatogonia. Further, there is no clear separation between a cortical and a medullary area as in most vertebrate groups. The presence of the deferens is not initially a reliable index of masculinity (D'Ancona, 1943).

An increase of the primordial germ cells is the first sign for recognizing a group of specimens with an early orientation toward the female sex, but such an increase takes place only in animals not less than 11 to 14 cm long (Rodolico, 1933 ; Mazzeschi & Vannini, 1936).

The distinctive characters remain unstable even during later stages. A minority of specimens show the first signs of orientation towards the female sex at lengths of 15 to 18 cm, but generally a female orientation is apparent at lengths of about 20 cm. The other specimens consist of males as well as animals with a delayed differentiation. Even at lengths exceeding 30 cm there are still individuals whose gonads show signs of intersexuality and can still deviate in both directions (Grassi, 1919 ; Mazza, 1932 ; D'Ancona, 1924, 1956 ; Rodolico, 1933).

The prevalence of growing oocytes is the essential characteristic of the female eel. It is only when auxocytosis and yolk formation occur that the cytological character of the oocytes becomes well defined.

The identification of the male eel becomes established with the appearance and the persistence of the deferens and of the cordonal arrangement of the germ cells. However, these characteristics may coexist with the presence of

the oocytes up to a certain stage. In such cases the animals are intersexes.

Thus, sex differentiation of the gonads can be recognized rather late in the life-cycle of the eel, with a remarkable variability as to size and age. Consequently the sex of eels can be defined with certainty only after they have turned silver or when, though maintaining the juvenile appearance, they have exceeded the maximum length which can be reached by males.

In the younger eels, with gonads apparently differentiated, sex reversal can still occur. There are also experimental data suggesting that silver eels may undergo sex reversal (Mazza, 1932 ; D'Ancona, 1956), though this occurrence cannot be considered as common.

The main manifestations of sex dimorphism in eels are as follows :

a. Different growth speed and extension.
b. Different migratory tendency.

It can be assumed that sex differentiation is under a genetic control, though this is not always well defined. The occurrence in some places of a larger proportion of males and in others of a larger proportion of females, might be due mainly to a different migratory tendency, but also in part to environmental influence. Males usually show a tendency to remain in coastal waters or in the lower reaches of rivers, whereas females tend to go upstream, sometimes quite a long way from the sea. At the edges of the distribution area of the species (Baltic, Upper Adriatic) females prevail, whereas in other littoral localities (west coasts of Italy, Latium and Tuscany) almost only males can be found (D'Ancona, 1958). This differential migratory tendency appears at an early stage, perhaps at that of the elver.

Results of experiments on elvers of different origins, reared in aquaria (D'Ancona, 1957) suggest that sex differentiation may be genetically controlled. However, such a genetic basis shows a surprising variability, due perhaps to a multifactorial determination. There is a transition through degrees of balanced intersexuality from animals with an early orientation towards the female sex to animals which are definitely males.

My own experiments (D'Ancona, 1956) suggest the possibility of a phenotypic sex deviation under the influence of experimental factors, acting in addition to genetic factors. The conclusions of Tesch (1928) and Gandolfi-Hornyold (1931, 1932) are in agreement with this but lack reliable documentation. Our experimental results show that high temperatures (D'Ancona, 1957 a, c) as well as crowding (Fidora, 1951) favour the differentiation towards the male sex.

Our research is also concerned with the effect of hormones (D'Ancona, 1956, 1957 b). We have seen that whereas testosterone does not effectively stimulate the male gonad, oestradiol induces meiosis and oocyte differentiation. A complication arises from the very slow effect of hormones in the eel. This is due in general to the relative inactivity of the endocrine system, at least in this stage of their life-cycle (D'Ancona, 1949).

THE METAMORPHOSIS FROM YELLOW TO SILVER EEL

The transformation from yellow to silver eel is another critical point during the eel's life. It consists first in a change of skin colour from yellow-green to

silvery or bronzy black and is accompanied by an increase in eye diameter. There are other changes which are listed as follows :

1. Increased thickness of the epidermis, derma and subcutaneous tissue, with accumulation of fat in the latter (Fosi, 1934) ; the number of muciparous cells in the epidermis becomes reduced, whereas that of the claviform cells, having also a secretory function, increases.
2. Change in pigmentation with an increase of melanin and guanine ; at the same time the scales and lateral line become more conspicuous.
3. Enlargement of the eye with change of the dioptric relations (D'Ancona, 1927 ; 1929 ; Feriani, 1954) and of the nature of the retinic pigments (Carlisle & Denton, 1959).
4. Change of the body flexibility and of locomotory behaviour, perhaps involving muscles and spinal column.
5. Change in the shape of the pectoral fins, which become lanceolate, and of the snout, which becomes narrower and pointed.
6. Increase of thyroid and pituitary activity (Evans, 1940 ; Callamand & Fontaine, 1942 ; Bernardi, 1943, 1948).
7. Greater gonadal development (D'Ancona, 1943).
8. Cessation of food consumption and reduction of the digestive tract.
9. Increased number of chloride cells in the gills (Colombo & Cecchini, unpublished) and change in osmoregulatory capacity.

Some of the above changes deserve a more detailed discussion.

The change in snout shape is not directly connected with the transformation from yellow to silver eel. This premise has always been based on the existence of large-headed and narrow-headed yellow eels (Ehrenbaum, 1930) though these have also been considered as separate races, if not as distinct species. According to our own observations, the big yellow eels with a particularly large head must be regarded as females with a delayed transformation. However, the change to silver eel always involves narrowing of the snout.

The enlargement of the eye with its consequent different dioptric relations and modified function is particularly interesting. The size-ratio between eyeball and lens is modified by this enlargement. The latter grow during the yellow eel stage but remain quite unchanged in the transformation to silver eel, when the main increase of the eye size occurs. During this transformation the number of retinic elements does not change ; in the enlarged retina the rods are spread and swollen. The blood-supply to the retina and the chorioidea is also greatly increased. The dioptric constants of the eye consequently change from a myopic condition in the young yellow eel to a hypermetropic condition in the big yellow and in the silver eel (Feriani, 1954). The retinal pigments have a higher density in silver eels and they change from a purple to a golden colour similar to that of chrysopsin (Carlisle & Denton). In this way silver eels acquire pigments similar to those of abyssal fishes.

All these changes do not indicate a degeneration of the visual organ, as Bertin (1951) has suggested. On the contrary the enlargement of the pupil, and the increase of the retinal density of photosensitive pigments facilitate, according to Carlisle & Denton (1959), a more efficient absorption of incident light. The eye of the silver eel seems, therefore to be better adapted to a new environment, i.e. to vision in more transparent and less illuminated waters.

The endocrine system is also modified in the silver eel. Together with increased development of the germ cells and of the whole gonad, during the transformation from yellow to silver eel, an increase in the activity of the thyroid and pituitary occurs (Evans, 1940 ; Callamand & Fontaine, 1942 ; Bernardi, 1943, 1948 ; see also Pickford & Atz, 1957).

Bernardi observes that this increased activity of the thyroid precedes the transformation. In silver eels there is an accumulation of the colloid and the activity of the thyroid seems to be reduced. On the other hand the pituitary is still strongly active. The main aspect of the transformation from yellow to silver eel seems to be connected with the activation of thyroid and pituitary from a lower functional condition.

The complexity of all these changes proves that in this transformation we are not dealing with the acquisition of a nuptial dress, which is normally transient. Instead there is an actual metamorphic transformation with a definite character like the metamorphosis from tadpole to adult frog or from ammocoetes to lamprey. Therefore, in the life-cycle of the eel we find two successive metamorphoses, the one from leptocephale to elver, the other from yellow to silver eel. I put this opinion forward in 1927, it was accepted later by Bertin (1951) and now it seems to be generally accepted.

According to Boucher-Firly (1935), Callamand (1943), Fontaine (1951, 1954) the transformation from yellow to silver eel is associated with hydration and loss of ions including chloride. Later in the marine environment eels show an increased concentration of ions in the blood particularly sodium and potassium. However, in these respects the course of events as yet has not been definitively shown and most probably they are very complex (Koch, 1949 ; Fontaine & Koch, 1950). Nevertheless, the transformation into silver eel and the consequent change of environment coincides with a change in osmoregulation (Black, 1957). This change is confirmed by the observation (Colombo & Cecchini, unpublished) that in silver eels kept in sea water the chloride-secreting cells of the gills contain a higher concentration of chloride than those of yellow eels kept in fresh water.

During the metamorphosis of the yellow into the silver eel, morpho-physiological characters peculiar to fresh water fishes become modified into those peculiar to marine bathypelagic fishes. The features and functions acquired are similar to those of related species living in a marine environment (*Conger*).

THE FINAL PHASE OF MARINE LIFE

The gonads of the silver eel before it leaves continental waters though more developed than those of the yellow eel, are still far from complete maturity.

In the male gonad the cordonal or tubular structure is more or less advanced. The germ cells are for the most part represented by spermatogonia ; nests of cells in the more advanced stages are scarce. However, in rare cases some small nests of spermatidia and also of mature sperms have been found (Grassi, 1913 ; Mazza, 1932 ; Rodolico, 1933 ; D'Ancona, 1943).

In the female gonad, oocytes are in different growth stages ; the bigger ones reaching diameters of 0·1 to 0·2 mm. Boucher, *et al.*, (1934), Fontaine

(1936), Tuzet & Fontaine (1937) and Schreiber (1935, 1936, 1937), by means of pituitary extract injections, obtained complete maturity of the male gonads in silver eels. The same result has not been obtained in the ovary. Carlisle & Denton (1959) by means of gonadotrophin injections have obtained oocytes of 0·4 mm in a silver eel and Bruun *et al.* (1949), by using chorionic gonadotrophins together with synthetic oestrogens obtained oocytes as big as 0·5 mm. From these observations it appears that silver eels migrating from river to sea—especially the females—are still far from complete sexual maturity. The finding of partial sperm ripeness already mentioned can be considered perhaps as an aspect of abortive prespermatogenesis similar to that occurring in other fish. The development of the few eels captured in the sea seems to be little more advanced than that of the common silver eel. Male eels captured in the Straits of Messina show only isolated groups of spermatidia or sperms and the females show oocytes no larger than 0·2 to 0·31 mm (Grassi, 1913).

Only one exceptional specimen fished in 1897 on the Danish coast and described by Schmidt (1906) had a well developed testis, full of ripe sperm. Another similar male was captured in the harbour of Hamburg (Schnakenbeck, 1949) but the gonads were not examined histologically.

I had the opportunity of examining two silver females captured in the Venetian lagoon, which had exceptionally big ovaries. However, on histological examination, the oocytes appeared no larger than normal, and the specimens did not seem to be notably advanced in sexual differentiation.

Little is known about the life of the eel after its return to the sea. Baltic eels recaptured in the Channel or in the Atlantic do not offer any further information. At Gibraltar, eels have never been captured. Tagging experiments made in the Mediterranean were unsuccessful.

We can, therefore, only advance a hypothesis on the final marine stage in the life of the eel, based on the characteristics they possess during the last period of their continental life.

In a single silver eel fished in the North Sea, Schnakenbeck (1934) observed the intestine filled with tissue which he could not identify with certainty, occlusion of the anus and sclerotization of the surrounding tissues. In other respects the eel seemed normal. The preservation of this specimen was not suitable for histological examination. Among four eels from the same provenience, occlusion of the anus occurred only in this specimen. Sclerotization of tissue might have occurred pathologically or by cicatrization following injury.

Berndt (1938) who has observed two specimens, believes that silver eels are subject to degenerative phenomena (histolysis, phagocytosis) of the digestive tube, particularly of the oesophagus and the intestine, whilst the stomach, especially in the pyloric region, appears normal. However, Berndt's documentation seems to me insufficiently conclusive.

I have never observed degenerative phenomena in silver eels such as those described by Schnakenbeck and Berndt. I have found only atrophy and functional inactivity, but they were not prejudicial to the soundness of the digestive organs, nor to their reactivation. Silver eels kept in fresh water aquaria after a period of fast can, in fact, refeed and recover weight.

Generally, both yellow and silver eels have an exceptional resistance and viability against inanition ; it is, therefore, reasonable to suppose that at sea silver eels can survive for a long time without feeding.

Ciaccio (1939), kept yellow eels in aquaria without food for more than 40 months. One of them after a fast of 49 months and 10 days had lost 80 per cent of its initial weight. These specimens showed atrophy and other alterations in the alimentary tract, but no spectacular degenerative phenomena (Fontana, 1941). In the same specimens the liver was the organ which was most adversely affected showing degenerative changes. Other organs, particularly the kidney, were better preserved (Gambaro, 1942, 1943).

Ciaccio (1944) also kept silver eels in fresh water under similar fasting conditions. Some of them lived under these conditions for more than three years. One eel after a fast of 45 months was re-fed and increased in weight.

It is reasonable to conclude from all these experiments that, although the silver eel may not be so resistant as the yellow, nevertheless it is in a good condition, well able, once in the sea, to endure a long period of fast and to spend a considerable time in reaching its spawning grounds with concomitant gonadal maturation.

Therefore, I do not believe that Dr Tucker's arguments, merely based on the observations of Schnakenbeck and Berndt, are sufficiently documented.

Our knowledge of the behaviour of eels in the final stages of their marine life is still scanty. We may presume, however, that they do not undergo further significant changes. Thus, eels from the Straits of Messina, apart from a more advanced development of the gonads, differ from the littoral and fresh water eel only in a heavier dermal pigmentation.

Further information relevant to this problem may be gained from an eel captured November 1948 at a fish farm on the Po River estuary (D'Ancona, 1951). Its length was 98·5 cm, but its weight was only 750 g. It was exceptionally slender and appeared to be quite aged ; on the otholiths we observed 22 rings. The eye was enlarged ; the colour was blackish bronze. Histological examination of the digestive tract revealed marked atrophy, but no particular degenerative phenomena. Histolysis was observed only in the liver and in some parts of the kidney. The ovary did not show signs of advanced development but the pituitary was exceptionally enlarged and showed signs of increased activity.

In my opinion, this specimen had been hindered from reaching the sea and was subjected to further transformations similar to those of the yellow to silver eel change, particularly with reference to the increased size of the pituitary. Thus the whole organization and physiology of the silver eel descending to the sea seems to be indicative of this adaptation to a new environment, namely deep marine waters.

There is little evidence that eels live after spawning, it is probable that the reproductive act concludes their life-cycle. This is the fate of *Conger conger* and other fishes.

The life-cycle of the European eel is certainly unique and still presents many problems. Further research should be directed to the solution of these problems.

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GENERAL DISCUSSION OF PAPER

Chairman : Professor Sir Solly Zuckerman, C.B., F.R.S.

D. W. Tucker : Unfortunately Professor D'Ancona has not allowed sufficient time for me to reply fully to the points raised. His paper is, however, almost entirely covered by Bertin (1956), Tucker (1959) and in the published correspondence between us (1959).

Professor D'Ancona's statement of the distribution of the American and European types of eel is not entirely correct ; Bruun (1937) cites a case of a glass-eel from North Spain with 108 vertebrae, and I have recollections of others.

The apparent vitality of silver eels on their departure from fresh water is immaterial to our problem ; what we are interested in is their condition by

the time they are a few hundred miles out to sea and in that context only the work which I have cited (1959) on eels which had made the Baltic run appears to be significant.

In postulating a temperature-effect upon numbers of myomeres and vertebrae in eels I cited experimental work upon *Salmo trutta* and critical reviews covering literally hundreds of populations of marine fishes as precedents ; to all this Professor D'Ancona opposes merely his personal opinion that such an effect could not operate in the case of the eel.

Since Professor Chester Jones has refused to give me any opportunity to study Professor D'Ancona's MS., I am not prepared to make any further comments in this place.

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REPRODUCTIVE ENDOCRINOLOGY IN CYCLOSTOMES AND ELASMOBRANCHS

BY

J. M. DODD, P. J. EVENNETT AND C. K. GODDARD*

Gatty Marine Laboratory, University of St. Andrews

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INTRODUCTION

Sexual reproduction in contemporary animals is a cyclic phenomenon and it seems reasonable to suppose that this must always have been the case, the period of the cycle in the early days being no doubt mainly controlled by external factors such as temperature and available food. It is, however, clear that a function as important as reproduction could not remain for long at the mercy of forces as fickle as those of a variable external environment, and a certain measure of intrinsic control must soon have become established. Thus the reproductive structures and processes of modern animals appear to be relatively independent of the external environment and to be controlled mainly by internal mechanisms. In the vertebrates for which the information exists, these are mainly of an endocrine nature, and take the form of complicated interactions between gonads, pituitary gland and secondary sexual characters, with the other endocrine organs playing less well defined roles. A great deal is known about these phenomena in mammals ; much less is known with certainty for the other vertebrates (Dodd, 1960a).

The animals which are the subjects of this paper are members of ancient vertebrate groups. As such, in addition to their own intrinsic interest, they

* Present address :—Department of Biological Sciences, University of New South Wales, Sydney, Australia

might be expected to make a significant contribution to any study on evolutionary aspects of reproductive endocrinology. In view of this it is remarkable that nothing has been known about pituitary-gonad relationships in cyclostomes until now and little indeed in elasmobranchs. It is the aim of this paper to assess from the results of previous work, and from new studies, the degree to which the development and physiology of the gonads and secondary sexual characters of these animals is under endocrine control.

CYCLOSTOMES

Lampreys and hagfishes are the only living representatives of the first vertebrate class to appear in the fossil record. Such animals are clearly of the highest importance in any comparative studies ; they have the additional recommendation so far as reproductive endocrinology is concerned that the free living species, at any rate, have a highly efficient reproductive mechanism, an unusually well-defined breeding cycle, well-developed secondary sexual characters and a pituitary gland having, apparently, all the regions which characterize this structure throughout the vertebrate series. It is clearly important to establish the nature of the physiological devices which control and synchronize the development of the gonads and secondary sexual characters and initiate spawning in these primitive vertebrates.

Lampetra planeri and *L. fluviatilis* are both dioecious species with well-developed gonads and secondary sexual characters. In the case of *L. planeri*, the ammocoete larva is the sole feeding stage ; it is generally believed to metamorphose in the late summer and autumn of its fourth year of life, with concomitant atrophy of the gut and ripening of the gonads. Spawning takes place the following spring and the spent animals die. *L. fluviatilis* is similar in all important respects, though it appears to have a short parasitic phase in marine or estuarine conditions immediately following metamorphosis.

The gonads

The gonad in both sexes is unpaired and median, and is suspended from the dorsal wall of the body cavity by means of a mesentery of connective tissue. In the mature animal the gonad occupies the greater part of the body cavity, the alimentary canal being completely atrophied. Cyclostomes are unique among vertebrates in having no intra-peritoneal genital ducts, though this condition is approached in some teleost fish. At sexual maturity a pore appears at the base of each urinary duct and the generative products, after being shed into the body cavity, escape to the exterior through these pores.

(i) *Testis*

The testis consists of a number of lobules united by connective tissue, each lobule containing several isolated ampullae. The ampullae are lined by a germinal epithelium which divides more or less synchronously throughout the gonad. *L. fluviatilis* ascends the rivers in the early autumn, and at this time only primary spermatogonia are present in the testes. These divide by mitosis and the testis grows. At the end of this phase of growth, spermatogonia are

transformed into spermatocytes and this stage appears to last for several months. The final stages of spermatogenesis take place suddenly in the early spring. These latter transformations occur close to the time of spawning and the ampullae become filled with mature spermatozoa. Prior to spawning the ampullae break down and the spermatozoa are liberated into the body cavity.

(ii) *Ovary*

There are no cytological studies available on the gonad of female *L. fluviatilis* though the gross appearance of the ovary has been described. It consists of a great number of ovarian follicles, all at roughly the same stage of development. Each follicle, when freed from its neighbours, is ellipsoidal, though tight packing gives the follicles an irregular outline in the intact gonad. Growth of the ovary is extremely slow throughout the winter, though when the water temperature rises in the spring a sudden burst of growth takes place, the eggs reaching their mature size by April (Fig. 2). When the eggs are ripe they are liberated into the body cavity and reach the exterior through two openings in the urinogenital sinus which become patent just prior to the act of spawning.

The secondary sexual characters

The external secondary sexual characters in both sexes of lampreys are the enlarged fins, the swollen cloacal labia, the curvature of the tail, and the appearance of an erectile 'penis' in the male. All the fins enlarge at the time of sexual maturity, the two dorsal fins become contiguous and a small post-anal fin appears ventrally in the female. The tail of the female curves sharply upwards, whereas in the case of the male there may be a slight downward curvature. At spawning time the cloacal labia enlarge in both sexes, due mainly to vaso-dilation, and a penis-like structure, which can be erected by influx of blood, appears in the male. The internal secondary sexual changes associated with maturity are limited to the pores which appear in the wall of the urinary sinus for the liberation of the genital products.

The pituitary gland (Pl. 2, Fig. 11).

It seems likely, in view of the recent work of Matty on *Myxine* (in the press) that all cyclostomes have a well-developed pituitary gland. In *L. fluviatilis* and *L. planeri* it takes the form of a flattened plate of cells closely attached to the floor of the third ventricle. It is divided into four distinct regions partially separated from each other by connective tissue. Pickford & Atz (1957) have termed the two anterior regions the pro-adenohypophysis and meso-adenohypophysis respectively. These regions are both broader and thicker than the meta-adenohypophysis which is a thin, narrow plate of cells lying under the neurohypophysis.

van de Kamer & Schreurs (1959) have assigned functions to the various regions of the pituitary on the basis of histological evidence obtained from a series of specimens of *L. planeri* taken throughout the life cycle. They found that the pro-adenohypophysis contained only one type of basophilic cell and that this gave a positive reaction for glycoproteins in the periodic acid-Schiff

test. The quantity of secretion in these cells was observed to decrease at spawning, and it was suggested that they produce the gonadotrophic hormone. The meso-adenohypophysis contains two types of cell : numerous chromophobes, possibly the source of the somatotropic hormone, and less frequent basophils containing granules which stain rose-violet by the Chrome-Haematoxylin-Phloxin method of Gomori. The basophils reach the peak of their activity at metamorphosis and are held by van de Kamer & Schreurs to be the source of the thyrotrophic hormone.

The experiments of Young (1935) showed that the lamprey pituitary contains a Melanophore-Expanding Hormone (MEH), the site of production being either the meta-adenohypophysis (pars intermedia) or neurohypophysis (pars nervosa). van de Kamer & Schreurs found that the production of secretion in the meta-adenohypophysis begins abruptly at metamorphosis when the animals leave the mud and take on their definite colour-pattern, and suggested on the basis of this evidence that MEH is secreted by this region of the pituitary. The neurohypophysis, particularly in the larva, contains droplets of neurosecretory material. This region is doubtless the source of the water-balance factor and other posterior pituitary principles, the presence of which in the lamprey was shown by Lanzing (1954), and Sawyer (1955).

Pituitary hormones and reproduction

Existing work on reproductive endocrinology in cyclostomes is confined to *Lampetra planeri* and *L. fluviatilis*, and much of it is discordant. Moreover it consists only of attempts to accelerate development of the gonads and secondary sexual characters by administration of hormone preparations and pregnancy urine ; hypophysectomy, although it has been carried out by both Young (1935) and Knowles (1941) has apparently never been investigated in the context of reproduction. The action of gonadotrophins on both mature and immature cyclostomes has been studied through the use of untreated pregnancy urine and extracts of mammalian anterior pituitary tissue.

Calvet (1932) injected ammocoetes of *L. planeri* with human pregnancy urine, and after two daily injections the ovaries of the only surviving specimen were said to be more mature than those of untreated animals. Calvet reports a better survival when the ammocoetes were bathed in one-third strength pregnancy urine for ten minutes on each of ten days ; six days after the last treatment the animals were killed. The oocytes were seen to be greatly enlarged, and the quantity of connective tissue reduced ; the secondary sexual characters remained unaffected. Damas (1933) injected adults of *L. fluviatilis* with pregnancy urine and described a striking effect on the gonads and secondary sexual characters. Both sexes were stimulated to produce fertile gametes more than a month earlier than the controls. However, in a later paper he was unable to repeat these results using, this time, animals on their autumn migration upstream, six months before the normal spawning time (Damas, 1951). It seems probable, then, that the 1933 experiments are unreliable, possibly because they were carried out at a time when even untreated controls are approaching maturity. In this connection it may be noted that Zanandrea (1956, 1957) has described neoteny in female ammocoetes

of *L. zanandreai*, in which mature eggs are visible through the transparent body wall of the larvae, and the secondary sexual characters of the sexually mature adults are present. In view of this Dodd (1960a) has suggested that experiments on endocrine physiology in lampreys should be carried out well prior to the normal time of onset of sexual maturity if the results are to be valid.

Young & Bellerby (1935) demonstrated the effects of injecting mammalian anterior pituitary extract into both larval and adult specimens of *L. planeri*. In neither case was there any visible effect on the gonads, though the secondary sexual characters were stimulated. Both the ammocoetes and the adults had swollen cloacal labia. In addition, the injected immature adults assumed the body form and fin conformation characteristic of sexual maturity. Since this work was carried out on animals in which the gonads were intact, it is impossible to decide whether the effects were direct, or were mediated indirectly via the gonads.

Knowles (1939) injected extracts of mammalian anterior lobe pituitary material into immature adults and ammocoetes of *L. fluviatilis*. The injections were carried out in January and February. This treatment had no effect on the fins of the treated animals, though there was an influx of blood into the cloacal labia which caused them to swell as they do at the time of sexual maturity ; there was no hypertrophy of the labial cells such as occurs at this time in nature. Knowles also obtained a break through between the body cavity and urinary duct which normally appears in March. In his experiments the connection became patent in January. Knowles was unable to find any unequivocal evidence of stimulation of the gonads of the treated animals in these experiments.

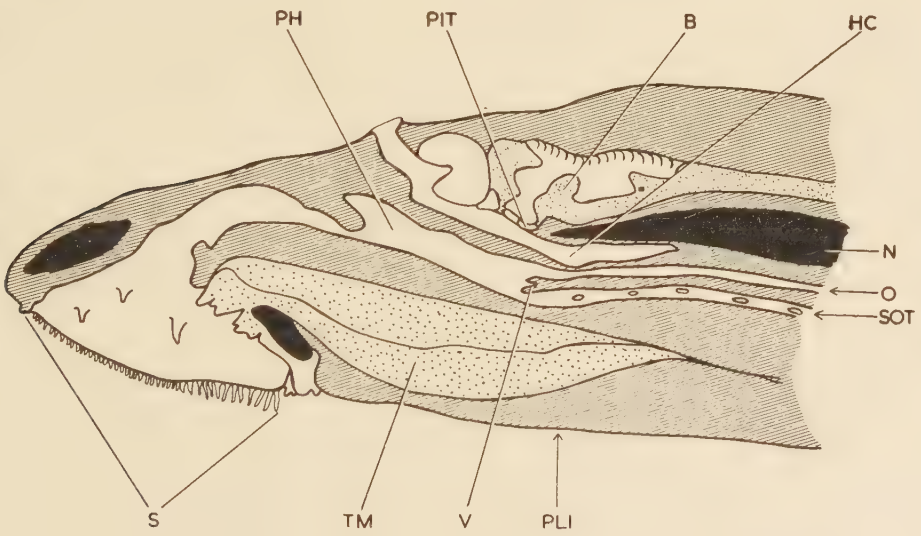

Fig. 1.—*Lampetra fluviatilis :* head and pharyngeal regions, sagittal section.
B, Brain; HC, Hypophyseal Canal; N, Notochord; O, Oesophagus; PH, Pharynx; PIT, Pituitary; PLI, Posterior Limit of Incision; S, Sucker; SOT, Suboesophageal Tube; TM, Tongue Musculature ; V, Velum.

It seems reasonable to conclude from this work that the findings of Calvet must be discounted, and that mammalian anterior pituitary lobe extracts are unable, under the conditions of the experiments reported above, to exert any effect on gametogenesis in ammocoetes or lampreys of either sex. There is no doubt, however, that injection of anterior pituitary extracts has a marked stimulatory effect on the secondary sexual characters of ammocoetes and adult cyclostomes ; hyperaemia of the cloaca and urinogenital papilla, fusion of fins, change in body form, and perforation of the urinary sinus have all been encountered as a result of treating larval and immature cyclostomes, and these features characterize the sexually mature animal.

Hypophysectomy (see Fig. 1)

The operation of hypophysectomy is difficult in *L. fluviatilis* because of the small size and inaccessible position of the pituitary. However, survival is good in the absence of fungal infections and since maturing lampreys do not feed, one of the main difficulties associated with maintaining animals in captivity does not materialize. A technique for hypophysectomizing lampreys was described by Young (1935) and this has been followed with only minor modifications. Anaesthesia is induced by immersion for about fifteen minutes in a solution consisting of one part tricaine methane sulphonate (MS. 222, Sandoz) in 2,500 parts tap water. The anaesthetized animal is attached to a specially designed board and a median incision is made in the ventral surface of the pharyngeal region from immediately behind the sucker to a point just anterior to the first pair of gills. The cut is now extended dorsad through the tongue musculature until it enters the pharynx. At the rear of the cavity thus formed lies the velum which marks the mouth of the sub-oesophageal tube. The septum dividing this tube from the oesophagus is incised backwards for a short distance to give improved access to the pituitary, and a cut made through the dorsal wall of the oesophagus into the hypophyseal canal. This cut exposes the pituitary which can be seen, with the aid of a binocular dissecting microscope ($\times 15$), as an oval, whitish patch in the roof of the hypophyseal canal. The pituitary is destroyed by a fine electric cautery needle and the resulting cavity plugged with sterile gelatine sponge. The edges of the wound are closed by a series of nylon stitches. On their return to water, the hypophysectomized animals soon resume active respiratory movements, and within a short time become strikingly pale (Pl. 1, fig. 3) and are no longer able to respond to a change of background.

Effects of hypophysectomy (Fig. 2 and Pl. 1)

Because of *Saprolegnia* infections only five hypophysectomised animals survived until April, at which time both the sham-operated controls and the normal animals in the tanks were sexually mature. Both sexes of sham-operated animals showed well-marked secondary sexual characters. In the males, the posterior dorsal fin was enlarged, swollen at its base and hyperaemic, and the gap between it and the anterior fin had disappeared. The cloacal labia were also enlarged, and the urinogenital papilla protruded between them as a sort of penis. Internally, the two genital pores had become patent

in the wall of the urinary sinus. The testis was much less firm than previously and was exuding a white fluid containing motile spermatozoa. The fins of the sexually ripe females became modified in a similar way to those of the males and, in addition, an anal fin developed behind the cloaca. The post-anal region of the tail showed a marked upward curvature, and the labia were swollen (Pl. 1, fig. 2). The ovary had ceased to be a discrete body and had become a fluid mass of eggs (Pl. 1, fig. 4).

The five hypophysectomized animals (one female and four males) showed no secondary sexual characters ; it was impossible to ascertain their sex from external features, and the two dorsal fins had no basal swelling and were separated by a gap of at least 1 cm. in lampreys of both sexes. Moreover the female showed no anal fin, the cloacal labia were not swollen, and no connections between the body cavity and the cloaca had become established. The body form of these animals corresponded to that of all lampreys in January when the operations were carried out. The gonads were immature, the eggs of the female were small and firmly held together with connective tissue, and on squeezing, the testes produced no milt (Pl. 1, figs. 3, 5 and 7).

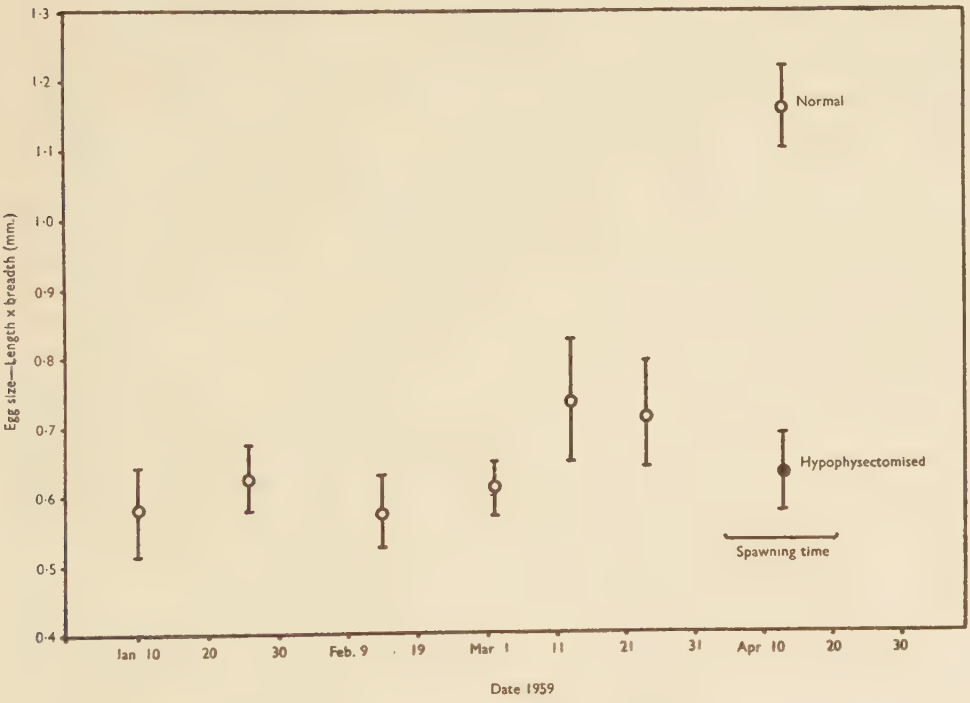

Fig. 2.—*Lampetra fluviatilis :* Graph showing growth rate of eggs and effects of hypophysectomy on egg size. The limits shown are standard deviations.

Samples of eggs were taken from normal female lampreys between January and April. The eggs are elliptical, and therefore both length and breadth were measured. The product of length × breadth, in millimetres, gave an

arbitrary index of egg size and the values obtained are shown graphically in Fig. 2. It can be seen from this that egg size in normal lampreys remains fairly constant until March, when it suddenly begins to rise until spawning time in April. As the graph shows, the eggs of the hypophysectomized female lamprey retained the typical January size ; hypophysectomy had evidently prevented further growth.

As has been stated, these lampreys were hypophysectomized in January, and at this time of year the testicular ampullae contain a high proportion of primary spermatocytes, though a few mitotic figures are still visible in testis sections, presumably indicating that some spermatogonia are also present and that these are still dividing. Since this work is of a preliminary nature, and because results have been obtained from only a few individuals, it is only possible to advance a tentative hypothesis. We suggest that pituitary gonadotrophins act mainly on the spermatogonial stages of the testis, and possibly also on those cells which are in process of transformation into primary spermatocytes. Once the cells have become primary spermatocytes, they can undergo all the subsequent maturation phenomena unhindered and become apparently normal spermatozoa. This hypothesis would account for the fact that fully developed spermatozoa are found in the testes of hypophysectomized lampreys, since these would be expected to develop from the primary spermatocytes present at the time of operation ; it would also account for the decreased amount of spermatozoa at the time of autopsy, since the spermatogonia believed to be present when the pituitary was removed would not be expected to develop further. It may be noted that this hypothesis gains support from similar work recently reviewed by Dodd (1960a), on other cold-blooded vertebrate groups and also from work reported below on elasmobranch fish. Experiments currently in progress on lampreys are designed to test the hypothesis further.

Steroid hormones and reproduction

There is no evidence available at the moment on the effects of gonadectomy on the secondary sexual characters of cyclostomes. The operation is difficult because of the size and extent of the gonads, though limited current success leads us to hope that data will soon be available on the effects of gonad removal.

In the absence of evidence which gonadectomy alone can provide, it is impossible to decide whether the injection of pituitary materials into larval and immature adult lampreys has a direct effect on the secondary sexual characters, or whether it stimulates the gonads to produce sex hormones which in turn act on the secondary sexual characters. Knowles (1939) has shown that the sex steroids testosterone and oestrone are capable of inducing the changes associated with sexual maturity in immature animals and this would appear to be indirect evidence in favour of the second view. Knowles has also shown that injections of either testosterone or oestrone (1 mg., and 250 i.u. respectively) caused the cloacal labia of immature adults of both sexes to become swollen and hyperaemic though the fins remained unaffected. These steroids were more potent in adults than were anterior pituitary preparations, and their effects were more marked in animals approaching sexual maturity.

Knowles also reported that both oestrone and testosterone advanced significantly the time at which the pores in the urinary sinus became patent in both sexes ; they were found as early as 29th January in treated animals, whereas they normally do not appear until March. These pores are never found in ammocoete larvae, in which the mesonephric ducts open into the rectum, and Knowles was unable to induce their formation by steroid injections, though certain mammalian pituitary preparations were highly effective in this respect.

ELASMOBRANCHS

Living elasmobranch fishes can be subdivided into two main groups, the Euselachii and the Holocephali. Nothing is known of reproductive endocrinology in the latter group, and little has been reported for the selachians until recently. These fish belong to an extremely ancient Class which has existed since the Devonian era. Thus they have been separated from the other vertebrates for more than 300,000,000 years, and several living genera, including *Scyliorhinus*, have existed since Cretaceous times, whilst others are still older, having originated in the Jurassic. It is clearly important to study the structure and function of the endocrine organs in these animals and to establish the chemical identity of their hormones.

Hormone specificity is an intriguing aspect of biochemical evolution and it has received a good deal of attention ; in spite of this the information at present available is too fragmentary to mean a great deal. The elasmobranchs, with their long evolutionary history, would appear to have special relevance in any comparative study on the biochemical evolution of hormones, and it should be possible to obtain from them evidence of biological specificity, if such specificity exists, against the time when the various hormones from the different groups will be chemically characterized.

Our present aim, at least so far as concerns the protein hormones, must be more modest and it will be sufficient as a first step to establish the structure and homologies of the various endocrine organs and to compare their functions and inter-relations with those known to exist in more recently evolved vertebrates.

The selachoid elasmobranchs (Pleurotremata), of which there are some 250 species, are the shark-like forms ; the batoids (Hypotremata) are less numerous and comprise the skates and rays. Many sharks attain a great size and experimental work on them would be impossible, but the Pleurotremata also includes the so-called dogfishes, of which the spotted dogfish, *Scyliorhinus caniculus* and the spiny dogfish *Squalus acanthias* are common members of the British fish fauna. *S. caniculus* is an excellent experimental animal, once feeding difficulties have been overcome, and most of our work has been carried out on this species (Goddard & Dodd, 1960). *Squalus acanthias* is much more difficult to keep under experimental conditions, though the fact that it is viviparous, and therefore of added endocrinological interest, makes it important that these difficulties should be overcome.

It may be noted that elasmobranch gonads and reproductive ducts are well developed structures in both sexes ; external secondary sexual characters are present in the male, and the group includes species which are oviparous,

ovoviviparous and viviparous*. Moreover, the pituitary gland is well developed, accessible and consists of three distinct and largely separate lobes. But in spite of these attractive features, little is known of the endocrinology of sex and reproduction in these fish. A good deal of new work on *S. caniculus*, the spotted dogfish, has recently been reported and discussed by Goddard & Dodd (1960), and this, together with previous work, is reviewed below. By way of introduction, since existing information on gonads, pituitary gland and secondary sexual characters of elasmobranchs is scattered and not extensive, these structures will be described.

The gonads

(i) *Testis* (Pl. 2, fig. 14)

Elasmobranch testes are paired structures which are suspended from the roof of the body cavity by mesorchia and, although they are somewhat variable in size, they are usually large. In *S. caniculus* they are elongated, subcylindrical structures which extend throughout the body cavity from its anterior end to the region of the rectal gland. Closely associated with the germinal tissue is a mass of lymphomyeloid tissue which partially invests the testes ; this is the so-called epigonal organ, the structure and functions of which have been discussed in some detail by Matthews (1950).

The ultimate units of the elasmobranch testis are the germinal ampullae, and these are frequently organized into larger units called lobules. In *S. caniculus* the testis has a zonate structure in which ampullae containing germ cells at a particular stage of differentiation occupy more or less parallel zones concentric with the so-called " tubulogenic zone " (Fratini, 1953) which occupies the mesio-ventral region of the testis. This arrangement results from the dorso-lateral migration of ampullae from the tubulogenic zone and the differentiation of their contained elements into spermatozoa during this migration. The histology of the testis of the basking shark, *Cetorhinus maximus*, has been excellently described and figured by Matthews (1950), and that of *S. caniculus* by Fratini (1953), thus there is no case for dealing here with testis structure in detail. However, since frequent reference will be made to a zone of breakdown, resulting from hypophysectomy, in that part of the testis bordering the spermatogonial ampullae, it is important to describe the salient features of this particular region. As already stated, the cells of the tubulogenic zone give rise to ampullae which at first contain only a few cells, though from the start they contain cells of two kinds. A lumen develops in each ampulla at a very early stage and one of the cell-types forms a single layered investment around it, whereas the other type occupies the peripheral regions of the ampullae. The inner cells have large, radially elongated nuclei and little cytoplasm. In consequence of the large nucleus and scarce cytoplasm these cells stain an overall blue with haematoxylin ; they are the fore-runners of the Sertoli cells and in later ampullae undergo a remarkable migration towards the periphery of the ampulla. In contrast, the germinal cells (primary spermatogonia), have rounded nuclei and abundant

* Viviparity is not treated in detail here since it is the subject of a later paper in this symposium.

cytoplasm and do not migrate. According to Fratini (1953), the Sertoli cells move towards the periphery of the early ampullae at the start of leptotene, the time at which the secondary spermatogonia become primary spermatocytes, their nuclei entering the prophase of the first meiotic division. Thus the Sertoli cells are a valuable diagnostic feature, enabling the status of the early ampullae to be accurately determined.

This transitional region passes distally into the zone containing typical primary spermatocytes which is characterized by germinal cells with large nuclei, usually seen in the protracted prophase of the first meiotic division. The Sertoli cells now lie at the periphery of the ampullae where they can be readily distinguished since their nuclei are pale after haematoxylin staining, in contrast with those of the germinal elements which stain deeply.

(ii) *Ovary* (Pl. 2, fig. 12)

The ovary varies a good deal in size and appearance in different elasmobranchs, that of *S. caniculus* being representative of the oviparous species. Matthews (1950) has described the ovary of *C. maximus* in detail and the difference between this ovary and that of *S. caniculus* may well be correlated with the fact that *C. maximus* is believed to be viviparous, though this is not known with certainty since no pregnant females of this species have ever been found. The single ovary in *S. caniculus* represents that of the right-hand side. In the sexually mature female it usually contains 10 to 20 large yolk-laden eggs, a varying number of eggs in early stages of vitellogenesis, a large number of small whitish primary oocytes and several yellow bodies which are probably corpora lutea vera and corpora lutea atretica (subsequently termed corpora lutea and corpora atretica). Each follicle appears to be limited by a granulosa and a two-layered theca. The histology of the corpora lutea has not yet been described for *S. caniculus*, though a detailed description of these bodies exists for other species (Samuel, 1943 ; Hisaw & Hisaw, 1960). The germinal tissue is usually embedded in a large epigonal organ, similar in all respects to that of the male.

The secondary sexual characters

Fertilization is internal in all elasmobranchs and the only external secondary sexual structures of the male are associated with intromission of sperm. They take the form of claspers which are intricate structures consisting of a pair of jointed, scroll-like, cartilaginous appendages lying at the margin of the pelvic fins and bordering the cloaca (Leigh Sharpe, 1920, 1921, 1922 ; Matthews, 1950).

In *S. caniculus* and most other selachoid elasmobranchs the claspers are finger-shaped and relatively short, even at maturity, but in batoids they are large pendulous structures in the mature male, and they may be heavily armed with modified placoid scales in the form of spines. Reports of copulatory behaviour in elasmobranchs are few, though it is well established that the claspers are inserted into the cloaca of the female at the time of copulation, serving to open it and thus exposing the lower ends of the oviducts.

Muscular sacs called siphons, lying just below the integument in the pelvic region, are associated with the claspers and it is believed that sea water is drawn into the siphons by muscular action at the time of copulation and, during the act of intromission, the water serves to flush the sperms into the oviducts of the female. It appears that relatively large amounts of sperm may be transferred and this remains viable in the folds of the shell gland of the oviduct and fertilises succeeding pairs of eggs, at least in oviparous species.

In female elasmobranchs, the pelvic fins have no claspers associated with them and there are no obvious secondary sexual characters, though the cloaca alters in character at the time of sexual maturity and the membranous hymens which close off the lower ends of the oviducts in immature females break down.

The immature cloaca is lined by a comparatively thin and dry epithelium and the cloacal vault is narrow and difficult to distend. At sexual maturity the lining becomes much thicker, is well vascularized and highly mucoid in character whilst the vault is extremely distensible and the oviducal openings are prominent and widely open.

The pituitary gland (Pl. 2, figs. 8, 9)

The elasmobranch pituitary gland consists of three well-marked regions and has associated with it the saccus vasculosus, a structure peculiar to fish, the function of which is in doubt, though it appears that it is not an organ of internal secretion. The elasmobranch neurohypophysis consists of an ill-defined zone occupying the cortical region of the neurointermediate lobe. In common with this structure throughout the vertebrate series, it appears to consist of a few parenchymatous pituicytes, together with the endings of an axonal tract which springs from the pre-optic nucleus and traverses the hypothalamus. Perks, Dodd & Dodd (1960) have described the structure and pharmacology of this region in *S. caniculus*. The adenohypophysis consists of three regions, of which the most posterior and dorsal is the pars intermedia component of the neurointermediate lobe ; intermedia tissue accounts for the greatest part of the bulk of this bulbous lobe. A tongue-like rostral lobe is partially embedded in the base of the neurointermediate lobe and projects forward from this site as far as the optic chiasma ; it is closely attached by connective tissue to the hypothalamic region, which it underlies. The third region of the elasmobranch pituitary is unique in its relations with the rest of the gland and with the skull. It has been called the " inferior lobes " (Baumgartner, 1915), the " pars ventralis " (Howes, 1936 ; Hogben, 1936) and the " inferior sac " (Charipper, 1937). The term " ventral lobe " has been used by most recent workers and this term is adopted here. It is a region which varies considerably in size with the state of sexual maturity of the fish and our evidence seems to show that most of the well-established functions of the vertebrate adenohypophysis reside in it. In selachoid elasmobranchs like the dogfish, the ventral lobe is surrounded by a thick connective tissue capsule and lies in a deep depression in the cartilaginous floor of the skull, being completely separated from the brain cavity by a covering of endocranium. It retains a tenuous connection with the posterior region of

the rostral lobe through a small hole in the endocranium. In sexually mature fish it is a large, diamond-shaped lobe of vesicular structure, the vesicles containing an abundant watery colloid. The long axis of the ventral lobe lies at right angles to that of the rostral lobe. In batoid elasmobranchs, the ventral lobe is placed much more superficially, there being no concavity in the cartilage. It therefore lies on the surface of the cartilage and is thinly covered by endocranium and usually pigmented. Moreover the interhypophyseal stalk is much more sturdy and is hollow.

The morphology of the elasmobranch pituitary and the homologies of its various lobes have received a good deal of attention (Herring 1908, 1911 ; Tilney, 1911 ; Baumgartner, 1915 ; de Beer, 1926 ; Comes, 1935 ; Ranzi, 1936 a, b ; Butcher, 1936 ; Lewis & Butcher, 1936 ; Charipper, 1937 ; Norris, 1941 ; Scharrer, 1952 ; Herlant, 1954 ; Dodd, 1960a). Much less is known of the histology, and apart from unpublished work of Hendry and Dodd, briefly reported below, no histochemical studies have been made. Comes (1935) studied the histology of the rostral lobe of the female *Torpedo* and described an irregular tubular structure consisting of chromophobe and eosinophil cells. The tubules were said to contain colloid secreted by the chromophobe cells bordering the lumina, and Comes reported that the amount of colloid increased during gestation. He concluded that these chromophobic cells produce gonadotrophin. Ranzi (1936 a, b) recorded changes in pituitary histology in female *Torpedo* and *Trygon* (both ovoviviparous) and *Mustelus* (viviparous) during gestation. He noted that as gestation proceeds, rostral and neurointermediate lobes show a general hyperaemia ; he makes no mention of the ventral lobe. There is also an increase in the amount of colloid in the tubules of the rostral lobe and the cells of the neurointermediate lobe are said to enlarge and to show increased eosinophilia during gestation. Howes (1936) described the morphology and histology of the pituitary of three species of *Raia* and reported that the rostral lobe could be divided into three regions on the basis of staining reactions. His attempts to find the homologues of these three regions in the mammalian adenohypophysis must be treated with reserve. Lewis & Butcher (1936) claimed that the pituitaries of *S. acanthias* and *Raia* can be divided into six lobes, one of which is the saccus vasculosus. The others are pars distalis and pars medialis of the rostral lobe, pars intermedia and pars neuralis (neurointermediate lobe), and pars ventralis.

Much the most detailed work to date on the elasmobranch pituitary is that of Norris (1941) who described pituitary morphology in fifty-one genera. He stated that the ventral lobe was highly variable in form, size and position and concluded that it " is frequently functionless and in some instances reduced to little more than a thin membranous sac. But in other forms its size, vesicular histological structure and abundant blood supply indicate an important function ". This statement must be interpreted in the light of the fact that Norris obtained his material in a preserved state from various parts of the world, and that it was presumably limited to the pituitary regions of the skulls of fish at very different stages of sexual maturity. In view of the findings reported below it is not surprising that the ventral lobes were very variable in appearance.

Recent literature on pituitary histology has relied a good deal on histochemical tests in interpreting the functions of the various cell types. In particular it has been claimed that cells secreting the glycoprotein thyroid stimulating hormone and gonadotrophins can be identified by their positive reaction to the periodic acid-Schiff (PAS) test and perhaps also the aldehyde-fuchsin (AF) test. There is no record in the literature of these tests having been applied to the elasmobranch pituitary. Our own results (Hendry & Dodd, unpublished) have shown that PAS-positive cells are invariably present in the anterior half of the rostral lobe and that these are the only cells which react positively in either the PAS or the AF tests. The colloid in the ventral lobe is intensely positive in both reactions, and it is possible that gonadotrophins are stored in it, but this positive reaction may merely indicate that the colloid itself is a muco-protein as, for example, is the colloid of the thyroid follicles.

We have no suggestion to offer as to the function of the PAS-positive cells of the rostral lobe. They are present from a very early stage in ontogeny and could therefore be thyrotrophs. However, we have a good deal of evidence that the thyrotrophic function of the dogfish pituitary resides in the ventral lobe. It is clear that more work is required before the functions of the dogfish pituitary can be interpreted with any confidence. Evidence to this end from pituitary removal is reported below.

Pituitary hormones and reproduction

The effects of removing endocrine organs from elasmobranchs are usually slow to emerge, hence a reasonably long post-operative survival period is an essential prerequisite in such work. Virtually all the early investigators, with the exception of Vivien (1940, 1941) were unable to ensure this, principally owing to inadequate feeding. In common with Vivien, we have resorted to force-feeding and have recently described methods for carrying this out which have enabled us to keep hypophysectomized dogfish alive and in good reproductive condition for more than two years (Dodd, Dodd & Goddard, 1959).

Early work on the elasmobranch pituitary is often difficult to evaluate because of this poor survival and also because details are rarely given of the parts of the pituitary which were removed. This point has special significance since the ventral lobe is never removed with the rest of the pituitary unless special precautions are taken and we believe this lobe to be the main controlling factor in elasmobranch reproduction.

There is a good deal of early work on pituitary control of colour change in elasmobranchs and several workers have investigated the pharmacology of the neurointermediate lobe. Orias (1932) found that pancreatectomy resulted in a diabetic condition in *Mustelus canis*, which was alleviated by hypophysectomy, whilst pituitary removal alone did not alter blood sugar level. Abramowitz, Hisaw, Boettiger & Papandrea (1940) confirmed these findings and produced evidence that the rostral lobe is responsible for this effect. So far as concerns gonadotrophic activity, there is evidence both from bioassay

work and from surgery that the elasmobranch pituitary plays a part in controlling the organs and processes associated with sex and reproduction.

Creaser & Gorbman (1939) attempted to identify gonadotrophin in the pituitary of *Squalus suckleyi*, but found that injections of four to sixteen glands did not induce ovulation in *Rana pipiens*. Goddard & Dodd (in Dodd, 1955) reported that injection of extracts of skate (*Raia batis*) neurointermediate lobe into immature mice produced an increase in uterine weight from 6·4 to 17·5 mg. This would appear to indicate the presence of gonadotrophin in this lobe, a most unlikely contingency. However, we have since found (unpublished results) that the neurointermediate lobe contains a heat stable substance which causes release of ^{131}I from the thyroid glands of mice under the conditions of the McKenzie assay for thyroid stimulating hormone (McKenzie, 1958), and we assume that its action is to release endogenous TSH, though this is not yet certain. In the light of these findings it is obvious that neurointermediate lobe extracts must be tested for gonadotrophic activity on hypophysectomized mice.

Vivien (1941) published details of experimental surgery on *S. caniculus*, but, unfortunately, his material and records were destroyed and no histological details could be given. This is especially unfortunate since two fish survived hypophysectomy for almost a year. In a preliminary communication (Vivien, 1940), he showed that hypophysectomy of male fish results in a progressive decline in the ratio of gonad weight to body weight. Vivien's later paper (Vivien, 1941) was written without access to records or material and is, in consequence, discursive rather than precise. However, important results are recorded in it. Hypophysectomy is said to block sexual development in young dogfish and to cause slow involution of the gonads of mature fish. Pituitary extracts and implants caused resumption of gonadal activity during the resting period of the sexual cycle (August–January) though, in females, injection or implantation was effective in this role only when it was carried out close to the time at which gonadal activity would normally be re-initiated. Extracts of mammalian pituitary material were also effective, though amounts up to two hundred, or three hundred times as much mammalian material were required before gonadal stimulation was achieved.

Carlisle (1954) reported that male dogfish will emit sperm at the urinogenital papilla as a result of light stroking of the belly region, whereas they do not respond in this way after a period of starvation, the length of which is not stated. However, Carlisle was able to induce the spermiation response in such starved specimens by injection of human chorionic gonadotrophin (110 i.u.), lactogenic hormone (50 i.u.), or mammalian anterior pituitary gonadotrophin (c. 100 i.u.).

Witschi (1955) tested elasmobranch pituitary material for gonadotrophic activity. He found that "shark" pituitary extracts gave both follicle-stimulating and luteinizing reactions, based on vaginal cornification and weaver-finch feather reactions respectively.

Hisaw & Albert (quoted by Dodd, 1955), found that ovulation in *Mustelus canis* is suppressed by hypophysectomy, though it can be re-initiated by implantation of homoplastic pituitary glands. They also reported that the

corpora lutea of this species are not dependent upon the presence of the pituitary either for their formation, or for their maintenance. The first three months of gestation are also apparently independent of the pituitary, though Hisaw & Albert were unable to say whether or not this also applies to the final eight months.

As already stated, some of the above work is difficult to assess in view of the few details which are given concerning the operation for pituitary removal. A cursory study of the elasmobranch pituitary shows how inaccessible the ventral lobe is in these fish and it is certain that this lobe invariably remains intact and functional after removal of the rest of the pituitary, unless special steps are taken to remove it. Our own work reported below appears to show that in *S. caniculus* at any rate, only the ventral lobe is concerned in gonadal maturation and, if we can extrapolate from this species to others, we must assume that " hypophysectomy " as reported by previous workers in other genera refers to total hypophysectomy and that the necessary steps were taken to remove the ventral lobe also, in spite of the fact that no special mention is made of this procedure. The work of Creaser & Gorbman must also be assessed on the basis of whether the ventral lobe component was included in their material. In this connection it may be noted that ventral lobes are extremely difficult to remove in routine collections of glands and, since the tough connective tissue capsule must of necessity be removed with the lobe, extraction of the lobe after acetone drying also presents special problems.

Hypophysectomy

Hypophysectomy has been carried out in a number of elasmobranch species by the following workers, among others : Lundstrom & Bard (1932), Orias (1932), Hogben (1936), Waring (1936, 1938), Abramowitz, Hisaw, Boettiger & Papandrea (1940), Vivien (1941) and Hisaw & Albert (1947). It is obvious from the description of the pituitary already given that the gland can be removed either entire or in part, and this is an important feature, which is rare among vertebrates.

(i) *Rostral lobectomy ; neurointermediate lobectomy*

The fact that the elasmobranch skull is cartilaginous, not bony, greatly improves the facility with which the pituitary can be removed. As a preliminary to the operation, the fish is anaesthetized in a 1 per cent solution of urethane (ethyl carbamate) in sea water, the anaesthetic bath being well aerated during the process. Anaesthesia is complete in a sexually mature fish of some 800 grams in twenty minutes. The fish, ventral surface uppermost, is then strapped to a specially designed board and the lower jaw maximally retracted. In this way the mucous membrane of the roof of the mouth is exposed and a median longitudinal incision is made in it, extending from the point at which the internal carotid arteries enter the skull to a point about 1 cm. from the margin of the upper jaw. Fairly profuse bleeding usually results, since the cut opens a venous sinus. Connective tissue strands run across this sinus, loosely attaching the mucous membrane to the cartilaginous

floor of the skull. These must be cut so that an area of cartilage can be exposed by retracting the cut edges of the mucous membrane. The approach to the cephalic pituitary (rostral and neurointermediate lobes) is through a rectangular trap door of cartilage which is made by incising three sides in such a way that the scalpel enters the cranial cavity, and so weakening the fourth side that it will act as a hinge. The exact location of the trap-door is critical if damage to the ventral lobe and its associated blood vessels is to be avoided. Fortunately, the point at which the internal carotid arteries enter the skull is an excellent landmark and the incisions must not come nearer this point than about 3 mm. They should extend backwards about 8 mm. on each side of the middle line and the flap of cartilage should be some 4 mm. in width at its widest point. Retraction of the flap exposes the rostral and neurointermediate lobes of the pituitary. These may be removed either together or separately, care being taken to prevent accidental removal of the saccus vasculosus since this adheres closely to the neurointermediate lobe. The operation just described leaves the ventral lobe intact, undamaged and functional.

(ii) *Ventral lobectomy*

In our earlier work ventral lobectomy was achieved through the intra-cranial approach described above. The ventral lobe is relatively easy to locate since it lies on the anterior face of the prominent inter-orbital ridge of cartilage ; it is destroyed by the use of a blunt seeker or an electric cautery. This technique for ventral lobectomy suffers from several disadvantages : the brain must be exposed ; the lobe cannot be seen, it must be located by touch ; it is difficult to avoid damage to the other pituitary regions, and complete removal is achieved in a relatively small number of cases. In view of these manifest drawbacks other techniques were tried. In the most successful of these the cranial cavity is not entered ; it involves the use of a dental drill to remove a triangular area of cartilage from the region immediately anterior to the carotid foramen. Careful drilling is continued until the connective tissue capsule of the ventral lobe is exposed ; this is then incised and the pituitary tissue removed by suction. Some bleeding is frequently encountered since the lobe is well vascularized and the carotid arteries and interorbital sinus are in the immediate vicinity. The cavity which results from the operation is packed with sterile gelatine sponge. The mortality resulting from this operation is negligible and post-mortem examination of the area by serial sectioning rarely reveals any ventral lobe tissue.

After all these operations the incised mucous membrane is rejoined by nylon sutures. Healing is very slow, but by the end of four months is usually complete. The pituitary region of every experimental fish is serially sectioned at autopsy and the results assessed in the light of the findings.

Effects of hypophysectomy in the male dogfish

(i) *Removal of ventral lobe* (Pl. 2, figs. 10, 14, 15)

The zonate structure of the dogfish testis has already been described and it will be evident from this description that the exact nature of any damage

inflicted on the testis by pituitary removal is particularly easy to identify since the various zones are discrete and serially arranged.

Throughout a long series of experiments abnormalities were occasionally seen in the spermatozoal ampullae, the spermatid ampullae, and the transitional ampullae lying between those containing spermatogonia and those containing primary spermatocytes. But there was little doubt that the effect of removal of the ventral lobe was to cause breakdown of this latter transitional zone. In virtually all fish in which the ventral lobe is shown at autopsy to be completely absent and which are examined more than six weeks after removal of the lobe, the entire zone shows signs of breakdown. In such fish the tubulogenic zone appears normal, as do the proximal ampullae of the spermatogonial zone. However, passing distally into those ampullae in which there are three or more rows of germ cells, the spermatogonia show signs of cytolysis and nuclear abnormality, though the Sertoli cells appear unchanged. The affected ampullae show a reduction in the number of nuclei and this may represent an effect on the ability of the spermatogonia to divide, though we found no direct evidence for this view ; they also show rosettes of nuclei which presumably mirror abnormalities in nuclear division. The ampullae shrink in size, and fragments of nuclear material are found scattered loosely among the cytoplasmic debris, the latter having the overall appearance of a syncytium· though a few cell boundaries are present. This precise picture has not been described in other cold-blooded vertebrates, though in the elasmobranchs we may well be looking at a slow motion picture of what in fact occurs in the other cold-blooded groups since everything happens extremely slowly in these fish. The fact that it is the early stages in spermatogenesis which are affected by pituitary removal is in general agreement with what is reported to happen in other cold-blooded vertebrates (see Dodd, 1960a, for a review). Several workers have suggested that pituitary removal stops mitotic division of spermatogonia. So far as dogfish are concerned, mitotic figures are so rarely seen, even in testes of normal animals, that we are unable to comment upon this possibility. However, it appears to be established that this, if it in fact happens in dogfish, is not the only effect since the early spermatogonial ampullae remain apparently normal, whereas those which might be assumed to contain secondary spermatogonia, or early primary spermatocytes, show the pronounced disintegrative changes just described. We tentatively conclude that the ventral lobe or, more specifically, the gonadotrophin(s) produced by it is essential for the normal transformation which occurs when a spermatogonium which, up to now has divided by mitosis, becomes a primary spermatocyte which will divide by meiosis.

In the vast majority of the fish which suffered ventral lobectomy these degenerative changes were well established six weeks after the operation, and with increasing time the expected sequelae were observed. Primary spermatocytes already established at the time of operation, and all succeeding stages of spermatogenesis, apparently developed in their normal fashion and in the testes of a fish twenty-two months after ventral lobectomy there were only spermatogonial and spermatozoal ampullae with the zone of breakdown still evident distal to the former (Pl. 2, fig. 15).

(ii) *Removal of rostral and neurointermediate lobes*

That the degenerative effects described above are specific to ventral lobectomy is shown by the fact that in no case of rostral lobectomy or of neurointermediate lobectomy, or of a combined operation, have we ever encountered any effects on the testis. For example, one fish which was autopsied fifty-five weeks after removal of both rostral and neurointermediate lobes showed completely normal testis structure.

In early experimental work we encountered degenerative signs in some spermatozoal ampullae and occasionally in the spermatid zone. These occurred in control fish as well as operated fish, and there was no correlation between the nature of the operation and the occurrence of damage. We believe that this type of abnormality can probably be adequately explained by inanition. In the early work, a loss of weight was frequently recorded during the experimental period due, presumably, to inadequate feeding. Since feeding has been mechanized and the amount of food increased these effects are rare.

Effects of hypophysectomy in the female dogfish

Throughout the period of the year during which it is possible to obtain our supply of dogfish from the west coast of Scotland (September–May), the ovaries of the mature females are large and well developed and usually contain 12 to 20 mature, heavily yolked eggs apparently ready for ovulation. In captivity these fish lay pairs of eggs regularly throughout this period at intervals of approximately three weeks. Most of our work has been carried out on fish which were known to have laid normal eggs not more than two weeks prior to the operation. Some 100 females have now been studied after partial hypophysectomy and the results are completely uniform. Unlike the males, no exceptions have been encountered.

(i) *Removal of ventral lobe* (Pl. 2, figs. 12 & 13)

No fish has laid eggs more than ten days after removal of the ventral lobe of the pituitary; such eggs as have been laid were undoubtedly either ovulated or about to be ovulated at the time of the operation. Indeed, on several occasions the upper third of the egg case laid by operated fish has been abnormal and this is believed to represent a traumatic effect of the operation on the shell-forming region of the oviducal gland. Control operated fish continue to lay eggs at approximately the same rate as unoperated specimens.

Ventral lobectomy has a profound effect on the ovary, an effect which appears to be in line with that reported for other cold-blooded vertebrates after pituitary removal. All eggs which are larger than 4 mm. at the time of operation, and in which vitellogenesis is well advanced, become atretic. The first signs of this process appear about three weeks after the operation; large, yolk-filled oocytes become flaccid and their follicles are markedly hyperaemic. As the process proceeds, the atretic follicles become bright yellow in colour and decrease in size. This process of follicular atresia following hypophysectomy has been noted by Hisaw & Abramowitz (1938, 1939) in *Mustelus canis* and *Squalus acanthias*, and the formation of corpora lutea

and corpora atretica has recently been described in detail by Hisaw & Hisaw (1960) in six elasmobranch species, three of which are oviparous (*Raia binoculata*, *Raia erinacea* and *Hydrolagus colliei*), two ovoviviparous (*Squalus acanthias* and *Squalus suckleyi*) and one viviparous (*Mustelus canis*). We have not yet studied the histology of atresia in *S. caniculus*, but there is good reason to believe that it will be closely similar to that described by Hisaw & Hisaw for these six representative species.

Although the onset of atresia is rapid after ventral lobectomy, its progress is relatively slow. Eleven months after the operation, the corpora atretica, resulting presumably from breakdown of the large eggs which were present at the time of operation, are still obvious structures, though small. By fourteen months after the operation the ovary consists of yolk-free oocytes of very small size, the remnants of corpora atretica and a large epigonal organ in which the germinal elements and corpora atretica appear to be embedded. In such a fish the oviducts show no obvious sign of shrinkage and retain their capacity to secrete the thread-like horny material by which the egg cases are attached to submarine structures. Again, no histological details are available, though these alone will show whether this degree of ovarian atresia has, in fact, left the oviducts completely unchanged.

(ii) *Removal of rostral and neurointermediate lobes*

As is the case in male dogfish from which the rostral or neurointermediate lobes, or both, have been removed, the reproductive processes and structures appear to be entirely unaffected. Several female fish have survived such operations for more than one year and the egg-laying pattern of these fish seemed completely normal, as did the ovaries and oviducts at autopsy. Thus results in the female are entirely in line with those reported for the male and the conclusion that the ventral lobe is the seat of gonadotrophin production in dogfish seems reasonably well established, though the evidence obtained from gland removal must be supplemented by replacement therapy before this proposition can be accepted without reservation. Such work is in progress.

Steroid hormones and reproduction

Castration and ovariectomy

Gonad removal in elasmobranchs is a severe operation because of the size of the incision required. Healing of the surface wound is slow, and, in spite of an efficient blood-clotting mechanism, there appears to be a steady leaching of blood cells through the clot and it is most likely that the animals die of anaemia. Our longest survival period for a castrate is one month and the secondary sexual structures were not obviously affected by the operation. Since there are no reports in the literature of either castration or ovariectomy, it follows that there is no direct evidence of a relationship between gonads and secondary sexual characters such as exists for all the higher vertebrate groups, including teleost fish.

There is, however, indirect evidence for such a relationship and this comes from implantation and injection of sex steroids.

Androgens and male elasmobranchs

Goddard & Dodd (in Dodd, 1955) have injected testosterone propionate into immature male skate (*Raia radiata*) at a dose level of 25 mg. per three days for forty-four days, and have shown that growth of the claspers can be stimulated. However, in spite of the massive dose of steroid, the average growth increment was less than 1 cm. There is no doubt that tissue sensitivity to steroids increases with age up to puberty and it is suggested that a greater effect would have been obtained on the claspers from this steroid if the recipient fish had been nearer to sexual maturity. Hisaw & Albert (1938) implanted pellets of testosterone into smooth dogfish and obtained a definite though small increase in growth. It may be noted here that batoid elasmobranchs are the material of choice for such work since their claspers show such a marked degree of growth and differentiation at the onset of sexual maturity.

It is not known whether the sex hormones play any part in the maintenance of the long coiled vas deferens or the various structures into which it differentiates distally.

Oestrogens and female elasmobranchs

The report by Wotiz, Boticelli, Hisaw & Ringler (1958) that oestradiol-17β can be extracted from the ovaries of the dogfish *Squalus suckleyi* is of great interest. This is the first recorded case of the chemical identification of a sex hormone in a lower vertebrate, though prior to this it had been widely assumed, on the basis of indirect evidence and by analogy with the mammals, that sex steroids were present in the gonads of these animals.

Hisaw & Abramowitz (1938) have shown that pellets of oestrogen, when implanted into female *Mustelus canis*, exert a stimulatory effect on the oviducts, and recent work of Dodd & Goddard (1960) has corroborated this in *S. caniculus*. Pellets of oestradiol were implanted intra-muscularly into female dogfish at different stages of sexual maturity, and these were found to have a pronounced stimulatory effect on the oviducts of certain of the treated fish ; secretion of the horny tendril-like material was especially stimulated. Rate of absorption of oestradiol was extremely slow, the most-stimulated female having absorbed only some 3 mg. in almost seven months. The marked stimulation produced by such a small amount of the steroid justifies the belief that this is a physiological effect. Dodd & Goddard also noted that sexually immature specimens were not stimulated by this treatment, and the oviducts of sexually mature fish showed no increased stimulation. The fish which were affected appeared to be those on the threshold of maturity, though vitellogenesis had not yet started in the most-stimulated specimen.

Steroids and sex-determination in elasmobranchs

The field of sex and sex-determination has recently been surveyed for all the lower vertebrate groups (Dodd, 1960b) but, since a significant contribution comes from work on elasmobranchs, this must be briefly mentioned here. The gonads in embryonic elasmobranchs (*Torpedo ocellata* and *S. caniculus*) are double, both in origin and in structure ; they therefore resemble Amphibia and Amniota and differ from cyclostomes and teleosts in this respect (Chieffi,

1952 a, b ; 1954 ; Thiebold, 1952, 1954). These workers have shown that testosterone propionate can inhibit the gonadal medulla of genetic males, though it has a stimulatory effect on Wolffian ducts and mesonephroi, and on the anterior segment of the Müllerian duct in genetic males, in which this duct normally atrophies.

Thiebold (1952, 1954) has shown that an injection of 50μg oestradiol benzoate will feminize the gonads of genetic males if injected into the yolk sac of embryos measuring 9–15 mm. The gonadal cortex in treated embryos is more prominent than in the controls and contains numerous gonia. Müllerian ducts and Wolffian ducts are also stimulated by oestrogen injection.

DISCUSSION AND COMMENTS

Studies on reproductive endocrinology in cyclostomes and elasmobranchs go far towards establishing the antiquity of endocrine controlling mechanisms in reproduction, and appear to show that gonad-pituitary relationships are fundamentally the same throughout the vertebrates. There is some evidence that the gonadotrophic region of the pituitary in these two classes may be more segregated histologically than is usual in the other vertebrate groups, though the teleost pituitary, consisting as it does of a single lobe with characteristic internal sub-divisions could easily be derived from the pituitary of either cyclostomes or elasmobranchs. No function can as yet be ascribed to the rostral lobe of the elasmobranch pituitary with any certainty, or to the pro-adenohypophysis of the teleosts, and the suggestion that this region of the pituitary in cyclostomes is the seat of gonadotrophin production is based only on histological and histochemical evidence. It may be that the presence of the pro-adenohypophysis is correlated with life in water, though such a speculation can only be assessed in the light of future research. There are strong indications in the work discussed above, and in unpublished results concerning thyroid-pituitary inter-relationships, that the elasmobranch ventral lobe is the important part of the pituitary in these animals and it may well be that it is the homologue of the entire adenohypophysis of the higher vertebrates.

There is no doubt that certain elements in the gonads of all vertebrates are under pituitary control and in cyclostomes and elasmobranchs it appears probable that these are the secondary spermatogonia and perhaps the transformation stages between these and the primary spermatocytes. The available evidence tends to show that the controlling hormone(s) is secreted by the ventral lobe of the elasmobranch pituitary, though it is impossible to say whether or not more than one hormone is involved, as is the case in mammals, and there is as yet little information concerning hormone specificity.

The position concerning sex hormones requires further clarification, especially in cyclostomes. So far as elasmobranchs are concerned, the demonstration of oestradiol-17β in the ovary of *Squalus suckleyi*, and the marked ability of implants of oestradiol to stimulate the oviducts of the female *S. caniculus* seems to indicate that these structures are under steroid control, as they are in other vertebrates. Information which can only come from gonadectomy is badly needed in both groups.

Viviparity is unknown in cyclostomes and we have never seen corpora lutea in *L. fluviatilis*, though Lyngnes (1936) has described them in *Myxine glutinosa*. Oviparity, ovo-viviparity and viviparity are all encountered in elasmobranchs, and corpora lutea and corpora atretica have been described in all species examined. But there is no evidence that the corpora lutea secrete progesterone, or that they help to maintain pregnancy in viviparous species. The simplest hypothesis at the present time is that these bodies are merely ova, or follicular debris, in process of resorption ; whether or not they play any part in the endocrine economy of elasmobranchs, or secrete progestins or oestrogens, must be the subject of future work. It may be noted that Pickford & Atz (1957) have given a fully documented discussion on the corpus luteum problem, so far as it concerns lower vertebrates, in their excellent monograph.

Existing evidence tends to show that the course of pregnancy in the viviparous species *Mustelus canis* is not influenced by pituitary removal, at least in its early stages, though nothing is known concerning the later stages and parturition. Such information would be extremely valuable, since it is clearly of the greatest interest to discover the point in the evolutionary series at which viviparity came under the influence of the pituitary.

However, in spite of all these unanswered questions, there seems no doubt that the gonads of cyclostomes and elasmobranchs and their secondary sexual characters are hormonally controlled and there is no reason to believe that the controlling mechanisms so far uncovered differ in any fundamental way from those obtaining in mammals.

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EXPLANATION OF PLATES

Plate 1.

Effects of hypophysectomy on *Lampetra fluviatilis* L.

Fig. 1.—Sexually mature male lamprey, April 13th, showing swollen cloacal labia with erectile penis, and enlarged, hyperaemic, closely apposed dorsal fins.

,, 2.—Sexually mature female lamprey, April 13th. Note that the body is swollen with eggs and shows enlarged cloacal labia and dorsal fins, post-anal pseudo-fin and upturned tail.

,, 3.—Hypophysectomised male lamprey, April 13th, displaying no secondary sexual characters. Note pallor. The appearance of hypophysectomized female lampreys is identical to that of the males.

,, 4.—Sample of eggs from a normal, sexually mature lamprey on April 13th. The eggs are loose within the body cavity.

,, 5.—Part of gonad of a hypophysectomized lamprey on April 13th. The eggs are small and still held together by connective tissue to form a compact ovary.

,, 6.—Section of the testis of a sexually mature normal lamprey. April 13th.

,, 7.—Section of the testis of a hypophysectomized lamprey, April 13th. Though spermatozoa are present which are apparently normal, they are fewer than in the normal animal.

Plate 2.

Pituitary glands of *Scyliorhinus caniculus* and *Lampetra fluviatilis :* effects of ventral lobectomy on the gonads of *S. caniculus.*

Fig. 8.—*S. caniculus :* pituitary gland in parasagittal section.

,, 9.—*S. caniculus :* pituitary *in situ* on ventral surface of brain. Ventral lobe not shown.

,, 10.—*S. caniculus :* T.S. part of testis to show zone of breakdown caused by ventral lobectomy.

,, 11.—*L. fluviatilis :* pituitary in parasagittal section.

,, 12.—*S. caniculus :* ovary of mature female 3 weeks after ventral lobectomy. Two follicles on extreme left are in early atresia.

,, 13.—*S. caniculus :* ovary 14 months after ventral lobectomy. One late atretic follicle visible : some ten more were seen on close inspection. Primary oocytes appear normal. Note large epigonal organ.

,, 14.—*S. caniculus :* T.S. testis of sham-operated specimen ; all zones represented.

,, 15.—*S. caniculus :* T.S. testis 22 months after ventral lobectomy. Note spermatogonial zone, zone of breakdown, large zone of spermatozoal ampullae and extensive epigonal organ.

(All Pl. 2 figures, except 9 and 13, from Goddard & Dodd, 1960.)

AF, atretic follicle (late) ; C, cartilage of floor of hypophyseal cavity ; CA, cartilage of base of skull ; E, early atretic follicle ; EP, epigonal organ; H, hypothalamus ; HC, hypophyseal canal ; M, meso-adenohypophysis ; ME, meta-adenohypophysis ; N, neuro-hypophysis ; NIL, neurointermediate lobe ; OC, optic chiasma ; P, pro-adenohypophysis ; RL, rostral lobe ; SPG, spermatogonia ; SP1, primary spermatocytes ; SP2, secondary spermatocytes ; SV, saccus vasculosus ; SZ, spermatozoa ; T, tubulogenic zone ; V, point of attachment of ventral lobe ; VL, ventral lobe ; Z, zone of breakdown ; III, third ventricle.

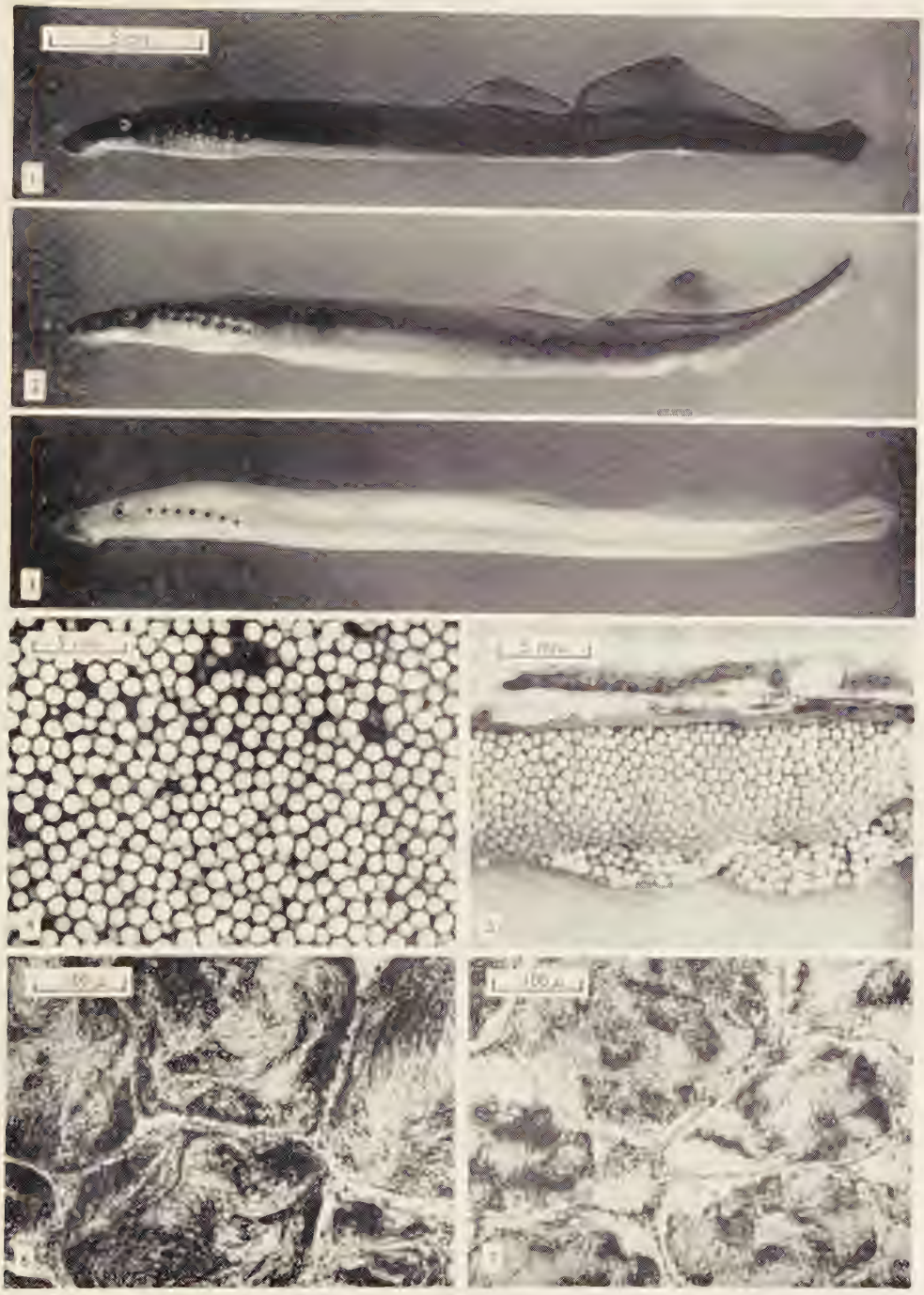
GENERAL DISCUSSION OF PAPER

Chairman : Professor I. Chester Jones

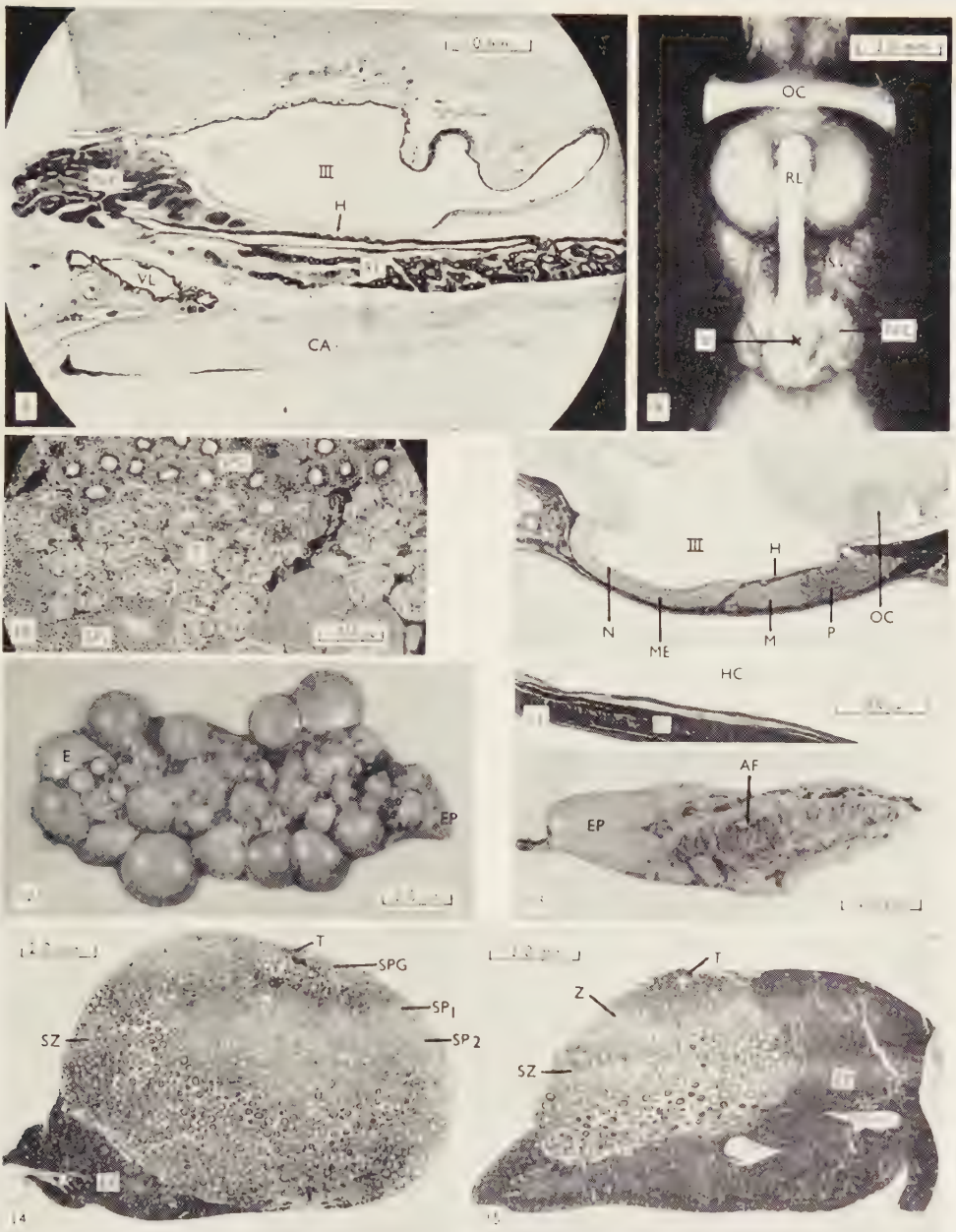
Sage : What effect, if any, does hypophysectomy have upon the interstitial tissue of the testis?

Dodd : We have not yet studied the histology of the testis in any detail, but this year's hypophysectomies will, I hope, answer your question.

Sage : van de Kamer and Schreurs (1959) and Lanzing (1959) have published accounts of cyst-like structures in the adenohypophysis of *Lampetra*



Effects of hypophysectomy on *Lampetra fluviatilis* L.



Pituitary glands of *Scyliorhinus caniculus* and *Lampetra fluviatilis*: effects of ventral lobectomy on the gonads of *S. caniculus*.

planeri and *L. fluviatilis*. van de Kamer has expressed the opinion that these cysts develop at the expense of the pituitary cells, while Lanzing regards them as extensions of the naso-hypophyseal canal. I have observed these cysts in all three regions of the adenohypophysis of *L. planeri*, both in laboratory stock and animals taken direct from the natural habitat. Cysts are present in approximately 25 per cent of the animals studied. Such animals appear normal. Cytological studies show that the cyst cells have similar staining properties and structure to the cells lining the naso-hypophyseal canal. This supports Lanzing's interpretation. I should be interested to know whether Dr Dodd has seen these cysts and how he interprets them.

Dodd : We looked for pituitary cysts but have so far found them in only one specimen of *L. fluviatilis* ; this had a single cyst in the meso-adenohypophysis. I wasn't aware that these structures were so common and I have no suggestions to offer as to their nature or significance. Have you seen cysts in *L. fluviatilis* as well as in *L. planeri*?

Sage : Yes.

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REPRODUCTION IN FEMALE BONY FISHES

BY

J. N. BALL

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INTRODUCTION

The animals coming within the scope of this review belong to the vertebrate classes Choanichthyes and Actinopterygii (Young, 1950). Leaving aside the recently resurrected coelacanths, not yet subjected to experimental investigation, the class Choanichthyes is represented today by the lungfishes of Australia, Africa and South America. Of the Actinopterygii, the vast majority are bony fishes *par excellence*, members of the huge order Teleostei, but the class in addition accommodates a few relict survivors of three once-important orders, the Holostei (*Amia*, *Lepisosteus*), Chondrostei (sturgeons) and Palaeoniscoidei (*Polypterus*, *Calamoichthyes*). Most of our information about the endocrinology of reproduction in bony fishes inevitably comes from work on teleosts, but quite extensive work has been done on the economically important sturgeons in Russia, and some information comes from *Amia* and from a lungfish *Protopterus*.

The great morphological diversity displayed by teleosts is reflected in the range of reproductive phenomena seen in these animals. Though predominantly dioecious and oviparous, self-fertilizing hermaphroditism is normal to some species, while viviparity has developed in several groups and attains high levels of specialization. Many, perhaps most, marine teleosts simply congregate in shoals at spawning time and broadcast the sexual products whole-

sale in the sea, but most shore and fresh-water fishes display some degree of courtship and spawning behaviour resulting in coordination of the orgasms. Copulation occurs in some species, and occasionally brooding and care of the young. The sexes are frequently indistinguishable externally except when the female is greatly distended with ripe eggs, but all degrees of sexual dimorphism are found, involving permanent or temporary differences of size, proportions and coloration and the development of various structural, physiological and ethnological differentiations, reaching an extreme in the Ceratioid angler fishes where the tiny degenerate male spends most of his life parasitically fixed to the free-swimming female who may be a thousand times his size (Norman, 1947 ; Hoar, 1955 ; Bertin, 1958 a, b). There is a striking contrast between those tropical forms which breed all the year round (Matthews & Marshall, 1956 ; Bertin, 1958 a ; Fryer, 1959) and the seasonal breeders of other regions. This latter group includes species with a short breeding season and others in which this period is prolonged over a large part of the year. Within the breeding season, in some species the individual fish releases all its eggs in a single spawning act, while in other cases the eggs are ovulated in batches at intervals over a long period (Gerbilsky, 1947 ; Kazansky, 1949 ; Pickford & Atz, 1957 ; Bertin, 1958 a). The majority of seasonal breeders spawn in spring and early summer, but some, like salmonids, are characteristically autumn and winter spawners.

Bertin (1958a) and Hoar (1955) give further examples of this great variety of reproductive habits, a variety which might reasonably be supposed to imply variations in the details of the endocrine mechanisms underlying the structures, behaviour and physiology associated with reproduction in these fishes. It is unfortunately true that our knowledge of these mechanisms is based on relatively few species, Dodd (1955), for instance, reckoning that reproductive endocrinology has been studied in fewer than fifty out of twenty thousand living species of teleosts, and in many of these, it may be added, only in a very superficial way. Thus generalizations, though they will be made, are tentative in this field and should be so regarded.

The recent excellent volume by Pickford & Atz (1957) clearly displays our current knowledge of fish endocrinology against the detailed background of information about mammals and birds. Overall, the same general processes appear to govern the reproductive physiology of fishes as form the classic corpus of vertebrate sexual endocrinology, though the details of these processes have been pursued hardly at all in the lower vertebrates. The material to be considered in this review therefore relates mainly to two organs, as in the higher groups, the pituitary gland and the ovary.

I. THE PITUITARY GLAND

A. *The pituitary and follicle stimulation*

The role of the pituitary in the development of the ovarian egg has been most convincingly demonstrated by the classical method of surgical extirpation of the gland followed by replacement therapy. In mammals, the growing ovarian follicle is dependent on pituitary stimulation only after a critical

stage, corresponding to the start of antrum formation (Hisaw, 1947 ; Simpson, 1959). In teleost fishes the beginning of vitellogenesis marks a similar stage beyond which the oocyte is dependent on pituitary gonadotrophins. After hypophysectomy small oocytes in the first growth-phase continue to grow up to this stage and then undergo atretic degeneration, while any oocytes past the critical stage and in the second (vitellogenic) growth-phase cease to grow and also become atretic (Vivien, 1941, 1952 a ; Buser-Lahaye, 1953). The process of atresia is apparently identical histologically with the spontaneous atresia commonly affecting oocytes in the second growth phase (Bühler, 1902 ; Wallace, 1903 ; Fraser & Renton, 1940 ; Stanworth, 1953 ; Ball, 1959) and with the histogenesis of the preovulatory corpora lutea described by Bretschneider & de Wit (1947). Compared with mammals, the effects of hypophysectomy develop slowly in fishes, so that atresia takes a matter of weeks or even months in *Gobius*, *Ameiurus* and *Xiphophorus*, and in this last fish the final para-infantile state is not reached until six to nine months after the operation (Vivien, 1941, 1952 a ; Buser-Lahaye, 1953). Finally the ovary contains only small first-phase oocytes, which may continue to grow slowly for some time in the absence of the pituitary (Olivereau, 1954). The effect of hypophysectomy on the ovary of a viviparous teleost is illustrated in Pl. 1, figs. 1 and 2.

Replacement therapy has been applied to hypophysectomized female fishes by Vivien (1939 a, 1941, 1952 a). In female rock gobies (*Gobius paganellus*), injections of cod pituitary material fifteen days after hypophysectomy restored the regressing ovary and induced second-phase oocyte growth. Homozoöic pituitary grafts or injections were similarly effective in causing follicular development in the inactive ovaries of long-term operated gobies, and in swordtails (*Xiphophorus helleri*) the involuting ovaries were restored by intra-ocular implants of adult pituitaries.

Supplementing this fundamental establishment of pituitary influence on oocyte development, there are numerous demonstrations that fish pituitary material will initiate or accelerate vitellogenesis when administered to intact fishes. Stoll (1957) achieved spectacular results by implanting pituitaries from maturing *Bathygobius soporator* into juvenile individuals of the same species, each fish receiving three adult glands. The ovaries of juvenile controls displayed many first-phase oocytes, with a few just started on vitellogenesis. One week after the implantations, the ovaries of the experimental juveniles were found to have been strongly stimulated. There was little increase in the overall size of the ovary, but histological examination revealed that some oocytes (presumably the most advanced at implantation) had grown enormously and were packed with yolk (Pl. 1, fig. 3). Most other workers in this field have given injections of pituitary material to experimental fishes. Donor and recipient may be of different species (Vivien, 1939 a ; de Azevedo & Canale, 1938 ; Cardosa, 1934 ; Hasler, Meyer & Field, 1939 ; Hasler & Meyer, 1942 ; Cherynshev, 1941 ; Rasquin, 1951 ; Tchechovitch, 1952 ; Kazansky, 1950, 1951, 1952), and material dried in acetone (Nishino, 1948, 1949 ; Ball & Bacon, 1954 ; Palmer *et al.* 1954) or preserved in absolute alcohol for up to

six months (de Azevedo & Canale, 1938) retains its potency, though Kazansky (1951, 1952) claims that acetone destroys follicle-stimulating potency. Vitellogenesis has also been induced in various fishes with doses of pituitary material from amphibians, elasmobranchs, reptiles and mammals, and with gonadotrophin from pregnant mare serum (PMS) and human chorionic gonadotrophin, though many negative results have been recorded using non-piscine preparations (Pickford & Atz, 1957). In general, the dose needed to get a response in this kind of experiment is greater than when fish pituitary material is employed; Vivien (1941) for example finding that fish pituitary material was one hundred or two hundred times as effective as that from mammals, weight for weight. The most likely explanation of this phenomenon is that the fish gonadotrophin to which the response of the fish ovary is adapted is slightly different from the non-piscine hormones administered in experiments. Pickford & Atz (1957) have suggested that throughout the vertebrate series the gonadotrophins contain certain physiologically active molecular groups, common to all the classes, but that these groups may be bound to other molecular parts in the nature of carrier proteins which differ phylogenetically. On this view, the differences in the carrier proteins would be responsible for such phenomena of phylogenetic specificity (Creaser & Gorbman, 1939 ; Pickford & Atz, 1957) as the high dosages of foreign hormones needed to obtain a response, and the lack of response to non-fish material noted by some workers. There is, perhaps, no absolute specificity, in the sense of a total inability to respond to foreign gonadotrophins, but examples of this relative specificity are numerous (see Pickford & Atz, 1957). The whole subject is closely connected with the formation of antihormones, perhaps antibodies to the species-specific carrier proteins, and this aspect has been discussed in general by Creaser & Gorbman (1939) and Simpson (1959), and in relation to fishes by Pickford & Atz (1957).

The site and mode of the follicle-stimulating activity of the pituitary is no better known in fishes than in mammals (Hisaw, 1947). The second growth phase, the only period in the life of the follicle absolutely dependent on the pituitary, is characterized by an enormous deposition of yolk during which the granulosa cells hypertrophy and multiply and are active in the passage of material into the oocyte (Ball, 1959) ; these same cells also undergo a dramatic change of behaviour during follicular atresia, whether spontaneous or following hypophysectomy, when they become digestive and phagocytic (Bretschneider & de Wit, 1947 ; Ball, 1959). Gonadotrophins may, therefore, primarily influence the behaviour of the granulosa cells. Another change seen during the second growth phase is in the theca, which becomes very much more vascular (Ball, 1959), an effect seen in the bitterling ovary following injection of carp pituitary extract (Bretschneider & de Wit, 1947). Whether this change and the granulosa cell hypertrophy are two distinct responses to gonadotrophins, or whether the granulosa hypertrophies in response to an increased follicular blood supply is not certain. However, the appearance of pronounced granulosa hypertrophy and hyperplasia before any detectable increase in thecal vascularity (Ball, 1959) does suggest that the two responses may be at least partly distinct.

B. *The pituitary and ovulation*

That ovulation ceases as a consequence of removal of the pituitary in fishes was demonstrated by Vivien (1941) who removed the gland from female gobies shortly before spawning and thereby prevented ovulation during the period when control fish spawned. He also showed that pituitary homo-injections would restore ovulation in operated fish, and that glands from other species were also effective in this respect.

Very many workers have been able to induce premature ovulation in bony fishes by administering fish pituitary material, usually as injections. One of the difficulties in this type of experiment is the provision of adequate controls, since in most fishes the spawning period is not sharply defined and it is common to find individuals spawning well before the main mass of the population. What is needed is a proper statistical comparison of experimental and control results, but unfortunately this has not been the usual procedure and some workers have dispensed with a control group altogether. Nevertheless, the mass of the evidence supports Vivien's demonstration that ovulation is directly dependent on the pituitary. Into this category comes the extensive work with food-fishes by Russian and Brazilian workers and an exhaustive review of the field is given by Pickford & Atz (1957). One point that emerges is that glands from any one species may be effective in provoking ovulation in any other bony fishes, but as with follicle-stimulation there is evidence of relative specificity correlated with phylogenetic relationship (Kazansky, 1940). The dosage relationships between different species have not been the concern of most workers, but Ramaswami & Sundararaj (1957) found that pituitary glands from the gravid catfish *Mystus* were as effective as homoplastic glands in causing ovulation in another catfish, *Heteropneustes*, whereas glands from ripe *Ophicephalus*, belonging to a different suborder, were ineffective at the same or greater dosage. There is, too, evidence that the female gland has a greater ovulation-inducing potency than that of the male, in catfishes, carp and sturgeons (Ramaswami & Sundararaj, 1957 ; Ball & Bacon, 1954 ; Barannikova, 1949 a), recalling the similar situation in Amphibia (Rugh, 1948). Ovulating potency is retained with little or no deterioration after preservation in absolute alcohol for up to at least two years, and perhaps longer (Fontenele, 1955) ,and in acetone for one year (Gerbilsky, 1940), and glands preserved in acetone retain at least some potency for ten years (Hasler *et al.*, 1950 ; see also Pickford & Atz, 1957). A point of some interest is that the Russian workers, largely concerned with sturgeons, have provided ample evidence that the pituitary-ovary link is essentially the same in these phylogenetically important animals as in the more familiar vertebrate groups.

Ovulation has been induced in bony fishes with pituitary material from elasmobranchs, amphibians, birds and mammals, and with PMS and HCG (Pickford & Atz, 1957 ; Ramaswami & Lakshman, 1959). Negative results have been common in this type of work, with indications of phylogenetic specificity (see above). Bony fish pituitaries have provoked spermiation and ovulation in various amphibians (Stroganov & Alpatov, 1951 ; Eliakova, 1954 ; Wills *et al.*, 1933 ; Ramaswami & Lakshman, 1959), and again negative results have been obtained (Atz & Pickford, 1954 ; Ball, 1959).

Nuclear changes in the oocyte just before ovulation have been described for sturgeons by Russian workers (Pickford & Atz, 1957) and for a loach by Kawamura & Motanaga (1950). These changes can be accelerated and their time relationships altered by pituitary treatment (Kazansky, 1950, 1954 ; Kawamura & Motanaga, 1950). Just before ovulation, too, the granulosa cells increase in height and their nuclei enlarge (Kazansky 1950 ; Ball, 1959), changes which have also been induced by pituitary treatment (Kazansky, 1950 ; Kawamura & Motanaga, 1950). The functional significance of these changes is not clear, but they suggest the possibility that pituitary gonadotrophins induce processes in the granulosa cells which result in ovulation. Both Kazansky (1950) and Kawamura & Motanaga (1950) observed histological changes suggestive of the accumulation of a secretion from these cells between the granulosa and the mature egg.

C. *The pituitary and the production of ovarian hormones*

The endocrine function of the fish ovary is discussed below. Concerning pituitary control of this function, we have the demonstration that hypophysectomy in female *Gobius paganellus*, in addition to its effects on the developing follicle, results in the persistent atrophy of the oviduct epithelium and of the genital papilla. Hypophysectomized individuals can be restored to complete normality with intramuscular or intraperitoneal implants of forty fish (*Gadus?*) pituitaries over a period of months (Vivien, 1939 b, c, 1941). Kinoshita (1938) found that injections of a pregnancy urine extract would elicit the female nuptial coloration in *Chloea sarchynnis* in the breeding season, when the presumptive ovarian endocrine tissue was well developed (§ III C), but was without effect after the breeding season, when there was very little of this tissue in the ovary. The development of spawning coloration in both sexes of the Pacific salmon *Oncorhynchus nerka* was accelerated by injections of pituitary suspension from *O. keta*, though mammalian anterior pituitary extract was totally ineffective (Palmer *et al.*, 1954). Coloration (nuptial, or sexual dimorphic) and accessory structures appear in general to depend on ovarian hormones (§ III B) but the possibility of peripheral actions of pituitary hormones—not necessarily gonadotrophins—cannot be ruled out. The work of Noble & Kumpf (1936) suggests a line of enquiry that should be pursued in this connection. These workers found that ovariectomy abolished the development of sexual activity in *Hemichromis bimaculatus*. Implants of frog pituitary into spayed individuals restored early courtship behaviour (in cycles shorter than normal) and caused growth of the atrophied genital tube ; but the genital tube was not affected by ovarian extracts. Thus this accessory structure may be at least partly independent of ovarian control, like the nuptial coloration of some birds (Witschi, 1955 ; Marshall & Williams, 1959). On the other hand, there is the possibility that as in the case of the bitterling ovipositor (§ III C), genital tube development after frog pituitary implants was part of a generalized " alarm " reaction, involving adrenal cortical activity and resulting from stress due to the injected foreign proteins.

The work of the group of Dutch workers led by Bretschneider & de Wit (1947) is relevant to this part and is considered in § III.

D. *Ovarian control of pituitary gonadotrophic function*

We have seen that pituitary control of ovarian function is well documented and of the general vertebrate pattern. It would be expected that the feed-back principle, well known from mammalian studies, should operate in fishes, so that the ovary by its endocrine activity would control in some manner the gonadotrophic action of the pituitary. In all probability this expectation will be borne out by future work, but critical evidence is lacking at this time. Cytological changes in the guppy pituitary after ovariectomy resemble those in spayed mammals which are plausibly interpreted as indicating accelerated gonadotrophin release due to the withdrawal of ovarian hormones ; this could well apply to the guppy (Sokol, 1955). Similar argument-by-analogy can be applied to the pituitary cellular changes seen in juvenile *Bathygobius* following the establishment of adult pituitary implants. The pituitary basophils degranulated gradually, parallel to enormous stimulation of oocyte growth, suggesting that ovarian hormone production was stepped-up by the extra gonadotrophin from the implants. The gradual degranulation of the pituitary cells possibly reflected inhibition of their production and storage of gonadotrophin (Stoll, 1957).

The effects of steroid hormones on the fish ovary are relevant to the whole problem. Oestrogens appear generally to inhibit the second growth phase of the oocyte, leading to follicular atresia (Bullough, 1942 ; Stanworth, 1953 ; Berkowitz, 1941 ; Tavolga, 1949 ; Egami, 1954 a). By analogy with the demonstrated situation in mammals (Greep & Chester Jones, 1950 ; Simpson, 1959), this ovarian inhibition probably results from the suppression of pituitary gonadotrophic output by the steroids. The experimental effects of the administered steroids, in all probability, is in line with the physiological action of the fish gonadal hormones, all the evidence being that these are steroids resembling the ovarian hormones of mammals (§ III).

One kind of experiment needed to establish the feed-back principle in fishes is that in which the effects on intact fishes of administering oestrogens alone and in combination with gonadotrophins are compared with the effects of similar treatment on hypophysectomized individuals. Egami (1954 b, c), though he did not employ hypophysectomized animals, has gone some way towards achieving this. He compared the effects of oestrone benzoate on intact *Oryzias* and *Misgurnus* with the effects of this steroid plus frog pituitaries, and with the effect of a combination of oestrone benzoate and mammalian gonadotrophins. Controls given the gonadotrophin alone showed slightly less ovarian development than those receiving only saline, but in both groups the ovaries grew and displayed vitellogenesis. Ovarian development was completely inhibited by oestrone benzoate, but this inhibition was attenuated if the gonadotrophins were given simultaneously ; vitellogenesis proceeded, indicating that the inhibitory effect of the oestrogen was due to suppression of the endogenous production of gonadotrophin.

In mammals and birds, gonadal steroids play an important part in inducing the release of the gonadotrophin responsible for ovulation (Fraps, 1955). Single injections of steroids have been found to cause ovulation in the loach, *Misgurnus fossilis* (Kirshenblat, 1952). Progesterone and DOCA were most effective and caused ovulation in all the fish treated. Methyl testosterone caused nuclear maturation in all fish, but only one out of ten ovulated. Ethinyl testosterone, testosterone propionate, oestradiol benzoate and oestrone were all negative. In contrast, Ramaswami & Lakshman (1958) could not induce ovulation in the catfish *Heteropneustes* with injections of steroids, though oestrogen, androgen and progesterone acted as synergistic agents when given with threshold doses of homoplastic glands. Much more work is needed to elucidate the significance of these results, especially since progesterone, testosterone and DOCA will induce ovulation in hypophysectomized amphibians (Shapiro, 1939 ; Kambara, 1954), but they do support the proposition that gonadal steroids may be an essential part of the ovulatory mechanism throughout the vertebrates.

E. *Non-gonadotrophic pituitary hormones and reproduction*

In addition to the gonadotrophins FSH and LH, the anterior pituitary in birds and mammals is known to elaborate a third hormone which plays a large part in reproductive activities. This hormone, prolactin, is essential in mammary-gland development and the secretion of milk, and of " crop-milk " in pigeons. It is connected with parental behaviour, and appears to have a luteotrophic effect in rats and perhaps in other mammals. It occurs in both sexes, but as yet there is no certainty about its rôle in the male (Beach, 1948 ; Cowie & Folley, 1955 ; Sturkie, 1954 ; Simpson, 1959). Until recently, the evidence for the occurrence of prolactin in fish pituitaries was suggestive rather than definitive, and was reviewed by Pickford & Atz (1957). Recently, Grant & Pickford (1959) have shown that teleostean pituitaries contain a factor which induces the water drive in the hypophysectomized urodele, *Diemyctylus viridescens*, an effect produced only by prolactin amongst mammalian pituitary hormones (Grant & Grant, 1958). Taken together with positive results obtained with fish glands in the pigeon crop assay (Lehrman, 1956), this constitutes strong evidence for the presence of prolactin in fishes. Whether prolactin plays any essential part in piscine reproductive physiology is uncertain ; results obtained in tests with *Hemichromis bimaculatus* (Noble, Kumpf & Billings 1936, 1938) are equivocal, and the widespread idea that these authors demonstrated prolactin to be responsible for parental behaviour in fishes is erroneous (Pickford & Atz, 1957). That prolactin plays an essential rôle in melanin synthesis in the melanophores of *Fundulus* is well established (Pickford & Kosto, 1957) and this may be the whole explanation of its occurrence in fishes. On the other hand, it is tempting to speculate that prolactin, acting as luteotrophin, may condition progesterone production by the fish ovary ; the possible distribution of both prolactin and progesterone throughout the vertebrates perhaps lends substance to this idea (Pickford & Atz, 1957 ; Dean & Chester Jones, 1959), though the presence of prolactin in the male remains unexplained.

Recently, Egami (1959) has found that a mammalian neurohypophysial extract would induce the S-bend spawning reflex in both male and female *Oryzias latipes*. The similar reflex in the related *Fundulus heteroclitus* is elicited by neurohypophysial hormones both in gonadectomized and in hypophysectomized fish (Wilhelmi *et al.*, 1955). This finding supports Egami's claim that the spawning reflex in *Oryzias* was a direct response to the injected extract. Egami also observed that any eggs already ovulated at the time of the injection, and lying in the ovarian lumen, were oviposited in the normal manner during the spawning reflex, presumably partly at least as a result of muscular pressure exerted on the ovary during the violent movements. The neurohypophysial extract did not bring about ovulation.

F. *The cellular origin and nature of fish gonadotrophins*

The whole subject of the sites of production of different hormones in the fish pituitary has recently been exhaustively reviewed by Pickford & Atz (1957). Assay experiments, involving the induction of maturation and ovulation in the loach *Misgurnus fossilis*, have demonstrated the concentration of gonadotrophic activity in the meso-adenohypophysis (Übergangsteil, transitional lobe, middle glandular region), both in carp (Kazensky & Persov, 1948) and in sturgeon (Barannikova, 1949 b). The meso-adenohypophysis appears to be the homologue of the mammalian pars anterior, and numerous studies of pituitary cytology in relation to gonadal state have indicated that gonadotrophin production in sturgeons and teleosts is the function of cyanophils (basophils) in this part of the gland, as in higher vertebrates (Pickford & Atz, 1957). Experiments on the guppy (*Lebistes*) distinguished two kinds of cyanophils in this region ; ovariectomy caused degranulation of one type (gonadotroph), and thiourea treatment produced degranulation of the other, the thyrotroph (Sokol, 1955). Histochemically, both thyrotrophs and gonadotrophs react positively to the periodic acid-Schiff (PAS) staining technique, like their mammalian equivalents, and all workers agree that the thyrotrophs are positive to the Gomori aldehyde-fuchsin (AF) technique. However, some find the gonadotrophs AF-negative, as in mammals (Barrington & Matty, 1955), while others claim that these cells are AF-positive (Atz, 1953 ; Sokol, 1955). This disagreement may reflect species differences, or may be due to the different fixatives and stains employed, the AF method being notoriously capricious (Barrington & Matty, 1955).

Fish gonadotrophins have never been extracted in any form remotely approaching chemical purity, and our knowledge of their chemical nature is inferential. That they are proteins, resembling the mammalian hormones, would be expected on general biological grounds. This is supported by the reactivity of the fish gonad to mammalian protein gonadotrophins, by the phenomenon of relative phylogenetic specificity encountered within the fishes (see above, parts A and B), by the cytological changes accompanying secretory activity in the gonadotrophs (i.e. loss of granules ; Pickford & Atz, 1957), and by the fact that chemical methods adapted to the separation of proteins have been applied successfully to fish pituitary material by Robertson & Rinfret (1957). These workers achieved partial separation of active

gonadotrophic and thyrotrophic material from pituitaries of Pacific salmon (*Oncorhyncus* spp.). Finally, there is the recent demonstration that proteolytic enzymes will destroy the ovulation potency of a fish pituitary homogenate (Ramaswami & Sundararaj, 1958). The fact that the specific secretory granules of the piscine gonadotrophs are PAS positive suggests that, like the mammalian hormones, fish gonadotrophins may be glycoprotein in nature, or bound to glycoproteins (*cf.* Pearse, 1952).

Though there is no satisfactory evidence that the fish pituitary actually produces more than one gonadotrophin, all our present information can be fitted into the usual scheme, involving two interacting gonadotrophins with functions similar to those in other vertebrates (Pickford & Atz, 1957 ; Simpson, 1959). A few experiments lend support to this concept as it applies to fishes, and these demand consideration at this point.

Most experiments in which fish pituitary material has been given to other vertebrates have yielded negative results, and to my knowledge only one worker, namely Kirshenblat (1949), has unequivocally induced follicular development in any other vertebrate using fish pituitary. He injected whole fish pituitaries into intact immature mammals and obtained follicular growth with antrum formation and ovarian hyperaemia in immature rats and mice. In one juvenile rabbit, injection of ten carp pituitaries was followed by marked hyperaemia of the ovaries and uteri and follicular growth ; in another juvenile rabbit, after a dose of twenty carp pituitaries, to these effects were added great increase in ovarian (three times) and uterine (six times) weight, and a two-fold increase in width of the vagina, effects produced, according to current concepts, by the combined action of two gonadotrophins FSH and LH (Evans & Simpson, 1950 ; Greep & Chester Jones, 1950 ; Simpson, 1959). Interpretation of these results requires some caution. The procedure could perhaps have caused the release of endogenous gonadotrophins in the mammals, due to the presence of releasing substances in the fish pituitaries. Such releasing substances would most probably be posterior pituitary polypeptides, as in mammals (Martini *et al.*, 1959). If this be a valid supposition, then there exists an interpretation of Kirshenblat's results other than that the fish pituitary contains two gonadotrophins resembling mammalian FSH and LH. However, both Kirshenblat and Vivien (1958) favour this latter hypothesis, which finds support in the failure of Pitressin, lyso-vasopressin and Pitocin to produce gonadotrophic effects in immature mice comparable to Kirshenblat's test animals (Martini *et al.*, 1959).

If we consider Kirshenblat's results with this later work in mind, we may perhaps accept the existence of FSH and LH in the fish pituitary, using the mammalian designates. We can then conclude that these factors sufficiently resemble their mammalian eponyms to act as effective mimics when given in adequate doses to laboratory mammals, but clearly further work, using hypophysectomized mice and rats, is required before a definite statement can be made.

However, there are other reasons for suspecting the presence of two gonadotrophins in the fish pituitary. Immature eggs are frequently ovulated following pituitary treatment (Pickford & Atz, 1957), indicating that follicular

rupture and final oocyte development are distinct responses to two different gonadotrophic situations. The difference may be purely quantitative (different amounts of a single factor) or partly qualitative (different relative amounts of two factors) ; the second possibility seems the more likely in the context of other work. There is the fact that the vitellogenic potency of the fish pituitaries declines as vitellogenesis is completed, well before ovulation (Kazansky, 1951, 1952 ; Stoll, 1957), whereas the ovulation-inducing potency does not decline until after spawning (Gerbilsky, 1940). There are also the results of Witschi (1955), who identified both FSH and LH in pituitary glands from salmon and *Lepisosteus*, using the rat vaginal cornification test and the weaver-finch test. This latter test appears to be specific for LH out of the mammalian complement of pituitary hormones. Furthermore, purified mammalian LH (dose 3–5 mg.) will induce ovulation in mature *Misgurnus fossilis* in both intact and recently hypophysectomized individuals, whereas FSH (1–50 mg.), like prolactin (5–50 mg.) and ACTH (1–25 mg.), is totally ineffective in the same test (Kirshenblat, 1956). Since, in addition, there is evidence for the incidence of both FSH and LH in amphibians (Wright & Hisaw, 1946), it seems justified to accept the presence of these two gonadotrophins in the fish pituitary. As Witschi (1955) has pointed out, one corollary of this is that the rôle of LH in ovulation is phylogenetically much older than the part it plays in the formation of the mammalian corpus luteum. Considering the phenomena of follicular growth and ovulation in fishes, one must conclude that two pituitary gonadotrophins would provide a better control of these processes than a single factor. Considerable evidence indicates that in most fishes eggs are not ovulated spontaneously when fully grown, but rather in response to particular stimuli provided by the environment or by the male partner (Pickford & Atz, 1957). There are many indications of more-or-less specific stimuli causing or influencing ovulation, presumably by stimulating the release of the appropriate gonadotrophin. For example, female *Tilapia*, when visually isolated continues to ovulate at a very low rate, but the sight of another individual, male or female, greatly increases the frequency of ovulation (Aronson, 1945, 1948). A two-component control system, with two pituitary gonadotrophins, appears to be necessitated by the need to retain fully-grown eggs in the ovary until the appropriate situation for their ovulation is presented.

II. ADRENAL CORTEX AND THYROID IN RELATION TO REPRODUCTION

A. *Adrenal cortex*

It now seems firmly established that the adrenal cortical homologue in teleosts is the " anterior interrenal " of Giacomini, groups of cells lying in the head kidney and usually associated with the cardinal veins and their branches (Rasquin, 1951 ; Stanworth, 1953 ; Chester Jones, 1957). It will be convenient in what follows to use the term adrenal cortex for these cells. Typical adrenocortical steroids have been identified in a wide variety of bony fishes (Chester Jones *et al.*, 1959), and the adrenal cortical tissue of *Fundulus heteroclitus* has been shown to produce corticosteroids from tagged progesterone *in vitro* (Phillips & Mulrow, 1959). Evidence that the piscine ovary elaborates

steroid sex hormones is reviewed below (§ III). As in mammals, there exist in fishes possibilities of mutual overlapping and mimicking in the endocrine functions of the adrenal cortex and the ovary (Chester Jones, 1957 ; Dean *et al.*, 1959), but at present we have little relevant evidence.

In mammals, normal gonadal function apparently requires the presence of the functional adrenal cortex. The literature has been reviewed by Burrows (1949), Zuckerman (1953), and Chester Jones (1957) ; we may note particularly the suggestion made by Mandl (1954) that corticosteroids enter into normal ovarian physiology at the cellular level. The possibility of a gonado-adrenal dependence in fishes is suggested by observations of adrenocortical hypertrophy and cellular indications of hyperactivity associated with gonadal development and spawning (Stanworth, 1953 ; Robertson & Wexler, 1957, 1959) ; the idea that this adrenocortical hyperactivity is occasioned by gonadal maturation as such, rather than by other collateral changes, is supported by the recent work of Robertson & Wexler (1959) on salmonids ; in these fishes, adrenocortical hyperplasia sets in with gonad enlargement, well before food reserves are depleted. It is not connected with migratory activity or with habitat changes, and the adrenocortical cells were found to be markedly hyperplastic in incompletely castrated male salmon when the small testicular remnant matured, one or two years after the operation.

Unfortunately, the results of experiments in which adrenocorticotrophin (ACTH) or cortical steroids have been given to intact fishes are impossible to interpret in terms of the direct action of corticosteroids on the gonad, because, as would be expected, the pituitary is affected by the treatment, both gonadotrophs and thyrotrophs undergoing marked changes. The matter is further considered in § III.

A sexual dimorphism in the adrenal cortex of some mammals is well known, the gland being generally larger in the female (Chester Jones, 1957). This difference seems to be dependent upon gonadal steroids ; oestrogens cause increase in adrenocortical size in the spayed female (spaying causes diminution of the gland) and in normal and castrated males, while testosterone diminishes the enlarged adrenal of the castrate male and the gland of normal males and females. That gonadal steroids may act in a reminiscent manner in fishes is indicated by Stanworth's (1953) results on intact *Gasterosteus aculeatus* ; injections of oestrone caused massive hypotrophy and hyperplasia of the adrenal cortical tissue, whereas testosterone had no effect or produced a slight decrease in the tissue. Progesterone caused a slight hypertrophy. Only oestrone caused lymphocyte depletion in the head-kidney and spleen, indicating hypersecretion of corticosteroids.

B. *Thyroid*

An increase in thyroid activity correlated with the spawning period has been recorded for several bony fishes (Buchmann, 1940 ; Heuts, 1943 ; Gerbilsky, 1947 ; Kuznetzova, 1947 ; Konradt, 1949 ; Olivereau, 1954 ; Barrington & Matty, 1954 ; Pickford & Atz, 1957). The many collateral changes associated with spawning makes interpretation of these observations difficult, and there are indications (Swift, 1955 ; Hoar *et al.*, 1955 ; Hoar,

1956) that this heightened thyroid activity during spawning may be causally related to the changes in physical activity involved in migration and spawning behaviour rather than to gonadal function as such. This idea would also accommodate cases in which depressed thyroid activity occurs at spawning (Lieber, 1936 ; Ivanova, 1954).

There is, in contrast, no reasonable doubt that normal thyroid function is necessary for ovarian development, as distinct from ovulation and oviposition. The developing eggs of sturgeons held in captivity degenerate, but are restored by thyroid injections (Kalashnikov, 1939 a, b ; Kalashnikov & Skadovsky, 1940, 1948). The degeneration was accompanied by marked alterations in the physiological properties of the blood, and probably should be taken as stemming from the role of the thyroid in promoting the healthy metabolic state necessary for gonad development. Certainly, the thyroid gland in sturgeons appears to be hyperactive during gonad development (Stroganov, 1952 ; Ivanova, 1953), and the same is true of teleosts (Swift, 1955, 1959 ; Konradt, 1949 ; Fortune, 1955). Experimental data confirm that lowering the thyroid activity in fishes (by antithyroid drugs or radio-iodine) results, *inter alia*, in inhibition of gonad development (Barrington & Matty, 1952 ; Scott, 1953 ; Pickford & Atz, 1957). Barrington & Matty were inclined to discount the attractive explanation that the thyroid exerted only an indirect effect on the gonad by controlling metabolism, on the grounds that a metabolic function of the piscine thyroid had not been demonstrated. However, we now have evidence that implicates the fish thyroid in metabolic regulation along familiar lines (Hoar, 1957 ; Pickford & Atz, 1957 ; Hopper, 1959), so that this explanation is acceptable.

III. THE OVARY

A. *Egg production*

The ovary in bony fishes consists essentially of numerous ovarian follicles embedded in a rather sparse connective-tissue stroma. In Dipnoi, Chondrostei, *Amia* and some teleosts, the ovary is a solid structure and the eggs are ovulated into the body coelom and passed to the exterior via oviducts which have free internal openings ; in *Lepisosteus* and most teleosts, the ovary is hollow and its lumen continuous with the cavity of the oviduct (Sedgwick, 1905 ; Bretschneider & de Wit, 1947 ; Romer, 1955 ; Gérard, 1958). Part of the epithelium covering the ovary is specialized as a germinal epithelium, and inpushings of this epithelium penetrate the stroma. Cells of the germinal epithelium differentiate into follicle (granulosa) cells and oocytes ; each oocyte becomes surrounded by a group of follicle cells (the granulosa layer) and develops to form, eventually, a mature egg. In this process, the oocyte passes through two phases of growth, previously mentioned in § I. In the first phase, growth is slow and cytoplasmic, in the second phase growth is rapid and involves the deposition of large amounts of yolk in the oocyte. At the same time the granulosa cells multiply to preserve a continuous layer around the oocyte, and stromal connective tissue is organised to form a theca outside the granulosa cells (Bullough, 1942 ; Bretschneider & de Wit, 1947 ; Stanworth,

1953 ; Ball, 1959). Pl. 1, fig. 4 illustrates the structure of the maturing ovary in *Coregonus*. The granulosa cells act as nurse or nutritive cells during vitellogenesis, an activity marked histologically by the appearance in them of lipoidal and other droplets, which pass into the egg through the pores of the oölemma or egg membrane (Ball, 1959). Ovulation of the mature egg is preceded by nuclear phenomena of maturation, and simultaneous changes in the granulosa cells have been described which may be concerned with the ovulatory mechanism (§ I B).

The developing egg is subject to specific endocrine influences. Injections of oestrone in the minnow (*Phoxinus*) and stickleback (*Gasterosteus*) greatly increase the rate of formation of young oocytes from the germinal epithelium. Progesterone was slightly less effective than oestrone in the stickleback and testosterone had no effect but was stimulatory in the goldfish (Bullough, 1942 ; Stanworth, 1953). Progesterone, androsterone, and oestradiol dissolved in the aquarium water also stimulated oocyte production from the germinal epithelium in the bitterling *Rhodeus amarus* (Bretschneider & de Wit, 1947). Saline injections had a minor stimulatory effect on oocyte production in the stickleback, possibly via the adrenal cortical tissue which was enlarged and apparently hyperactive following this treatment and, indeed, after most experimental interference (Stanworth, 1953). Because of this, Stanworth was inclined to regard oocyte formation under these experimental conditions as being induced by corticosteroids secreted at an enhanced rate (see below, § III B). However, the regular natural increase in oocyte production during the post-spawning period in many fishes suggests that ovarian hormones may themselves enhance the process, as in mammals (Burrows, 1949), and that the effects of the steroids given to fish in experiments may be partly direct and only partly mediated via the adrenal cortex. A most interesting finding is that cod pituitary extracts significantly depressed oocyte production in the stickleback during the post-spawning period, though the adrenal cortex was enormously stimulated (Stanworth, 1953). The pituitary extract was gonadotrophically active, and there is here the possibility that gonadotrophins may actually depress the formation of young oocytes while stimulating vitellogenesis in second phase oocytes (§ I A). The idea accords with the distribution of activity in the germinal epithelium in the yearly cycle. In *Cottus bairdii* (Hann, 1927), the minnow (Bullough, 1939, 1942), bitterling and *Zoarces* (Bretschneider & de Wit, 1947), stickleback (Stanworth, 1953), trout and *Coregonus* (Ball, 1959), though oocyte production probably continues at a slow rate throughout the year, the process is enormously accelerated during the post-spawning period, when the gonadotrophic content of the pituitary is at its lowest (Gerbilsky, 1940 ; see § I). Oocyte formation and vitellogenesis may thus be regarded as broadly alternating phases of ovarian activity ; the former activated by steroid hormones, the latter by pituitary gonadotrophins. If gonadotrophins oppose the stimulation of oocyte formation by ovarian hormones, it is understandable that oocyte production is not enhanced during the breeding season when, to judge from the state of secondary sexual characters, ovarian endocrine activity must be at its height.

B. *The ovary as an endocrine organ*

Surgical removal of the ovary in actinopterygian fishes has amply demonstrated the endocrine function of this organ. The items shown in this way to be dependent upon ovarian hormones include the seasonal modifications in liver size and cytology characteristic of female *Oryzias* (Egami, 1955), the genital papilla in *Tilapia* (Aronson, 1948), *Hemichromis* (Noble & Kumpf, 1936) and *Oryzias* (Yamamoto & Suzuki, 1955), ovipositor growth in the bitterling *Acheilognathus* (Tozawa, 1929), female coloration in *Tilapia* (Aronson, 1948) and *Amia* (Zahl & Davis, 1932), and the female arrangement of certain haemal spines in *Xiphophorus* (Vivien, 1952 b). Most commonly, however, in fishes it is the male that shows pronounced modifications of juvenile structure or coloration, and castration results in a reversion to the indifferent or female type ; in the female, differing little if at all from the juvenile, removal of the gonad usually has no obvious effects (Okada & Yamashita, 1944 a, b ; Hopper, 1949 a, b ; Pickford & Atz, 1957). Ovariectomized *Mollienesia latipinna*, for example, look exactly the same as intact females, and are courted vigorously by males, even in a choice situation in competition with normal females (Ball, 1959). Nevertheless behavioural changes have followed from ovariectomy in some species : spayed *Xiphophorus* females dropped down their social hierarchy (Noble & Borne, 1940), and ovariectomy abolished sexual behaviour in female *Betta splendens* and *Hemichromis bimaculatus* (Noble & Kumpf, 1936) ; in both species the behaviour was restored by ovarian extracts from an unspecified source. An ovarian influence on general metabolism is suggested by liver changes following ovariectomy (Egami, 1955), and by rapid and enormous deposition of perivisceral fat in spayed *Gasterosteus* (Bock, 1928) and *Mollienesia* (Ball, 1959).

These experiments establish an ovarian endocrine function quite definitely, but tell us nothing about the chemical nature of the ovarian hormone or hormones. For many years there has been evidence that the fish ovary contains substances with oestrogenic actions on laboratory mammals ; some slight activity resides in extracts of fish eggs (Sereni *et al.*, 1929 ; Donahue, 1949), as well as in extracts of whole ovaries (Fellner, 1925 ; Fleischmann & Kann, 1932 ; Weisman *et al.*, 1937 ; Donahue, 1941), and is present in ether and acetone extracts. Recently, D'Ancona *et al.* (1955) induced feminization in genetically male tadpoles of *Rana esculenta* by immersing them in suspensions of dessicated powders of ovaries or eggs of rainbow trout, an effect that could be produced by weak doses of oestrogens ; strong doses of oestrogens in this frog are androgenic (Gallien, 1955). Out of the welter of effects induced by dosing fishes with mammalian or synthetic oestrogens (Hoar, 1951, 1955 ; Dodd, 1955 ; Pickford & Atz, 1957), there are some whose dependence on piscine ovarian hormones has been established by surgery [growth of the genital tube in spayed *Tilapia* (Aronson, 1948), induction of female liver size and cellular structure in several species (Kobayashi, 1953 ; Egami, 1955 ; Oguro, 1956), haemal spine formation in *Xiphophorus* (Vivien, 1952 b ; Mohsen, 1953)] and others whose dependence on the ovary is indicated by synchrony between their development and the ovarian cycle ; these include

the induction of female-type skeletal elements in *Lebistes* (Scott, 1944) and in *Oryzias* (Egami & Ishii, 1956), development of nuptial coloration in *Chloea* (Kinoshita, 1938), the development of a connection between the oviduct and the exterior in *Phoxinus* (Bullough, 1942), and hypertrophy of the peritoneal epithelium in trout (Ashby, 1957). This last case serves to introduce the anomalous results often obtained with steroids in fish : Ashby, working with young trout, found that while oestradiol was the most effective inductor of peritoneal hypertrophy, testosterone also had a strong effect, though such hypertrophy is virtually absent in adult male trout. Alcohol also caused peritoneal hypertrophy, possibly through a " shock " effect acting via the stimulated adrenal cortical tissue. Testosterone, and not oestradiol, caused spayed and intact female *Xiphophorus* to ascend their social hierarchy (Noble & Borne, 1940), and oestradiol benzoate produced ovarian degeneration and growth of a male-type gonopodium in female *Platypoecilus*, though oestradiol did not affect gonopodial development (Tavolga, 1949). Enlargement of the genital papilla in *Hyborhynchus*, a female character, was induced much more effectively by testosterone propionate than by oestradiol (Ramaswami & Hasler, 1955). Oestradiol benzoate given to spayed *Tilapia* did not restore the female opercular coloration, and given to intact females actually caused the disappearance of this character, while the regressed genital tube in spayed individuals was restored by both testosterone propionate and oestradiol benzoate (Aronson, 1948). These and similar facts have led to the view that the hormone or hormones of the fish ovary may be different from those of mammals (Hoar, 1955 ; Pickford & Atz, 1957). Emmens (1959) has suggested that though oestrogens exist in fishes they may not be steroids. However, Dean & Chester Jones (1959) have recently applied chromatographic methods to ovarian extracts of the African lungfish *Protopterus annectens*, and by this means have demonstrated the presence of oestriol, oestrone and a trace of oestradiol-17β. The extract produced a positive response in the mouse uterine weight test. These workers also separated and identified progesterone chromatographically in the lungfish ovarian extract. These important results allow us to say definitely that the fish ovary produces substances that are chemically and biologically oestrogens. Which particular oestrogen predominates in different fish, and which is most active in its effects on terminal organs remains to be determined ; variations in these factors might well explain some of the anomalous results obtained with mammalian and synthetic steroids in fishes. Oestriol was overwhelmingly predominant over oestrone in the lungfish (Dean & Chester Jones, 1959), and very little oestradiol was present. This contrasts with mammals, in which oestradiol seems to be the principal steroid actually formed in the ovary, oestrone and oestriol being regarded as secondary or excretory products (Burrows, 1949 ; Emmens, 1950).

C. *Discussion : Sites of hormone production in the fish ovary*

With the work of Marshall & Lofts (1956) and Lofts & Marshall (1957), the cellular site of endocrine activity in the teleostean testis was well established, interstitial cells comparable to the mammalian Leydig cell being found in some fishes and lobule-boundary cells, derived from fibroblasts, in others.

Unfortunately, no such certainty exists in the case of the female, though the possibilities in this field have been extensively discussed during the past ten or twelve years. We are still without decisive evidence of the endocrine function of any type of cell or structure in the ovary of bony fishes.

Histologically, the possible structures are few. The theca of the developing follicle consists of collagen fibres and fibroblasts that at no time contain lipid droplets or cholesterol (unlike the lobule boundary cells of the male) and never approximate to the appearance of the probably oestrogenic cells of the theca interna of mammals and birds (Corner, 1938 ; Hisaw, 1947 ; Burrows, 1949 ; Marshall & Coombs, 1957), while the ovarian stroma is sparse, consisting of fibroblasts, fibres and blood vessels. At one time the granulosa cells were thought to produce hormones in mammals, but this now appears improbable (Corner, 1938 ; Burrows, 1949). In teleosts, these cells *in situ* seem never to contain cholesterol or to be secretory, and the lipid droplets they display in the maturing and ripe follicle appear to be passed via the oölemma into the egg (Rasquin, 1951 ; Ball, 1959). Perhaps it is unlikely that these nurse cells when actively engaged in passing material into the egg should simultaneously elaborate steroid hormones.

The work of Bretschneider & de Wit (1947) has come under heavy fire in recent years (see Pickford & Atz, 1957), and rejection of the bitterling ovipositor test as an endocrinological technique seems to have led to the summary dismissal of all the hypotheses elaborated by the Dutch workers in relation to this test. These hypotheses were supported by evidence that was only briefly presented and, as it now seems, almost certainly misinterpreted. Nevertheless, their central idea—that the endocrine cells of the fish ovary are derived via follicular atresia from granulosa cells—deserves more consideration than it usually receives, and is in keeping with the evidence from fishes and from other vertebrate groups. It seems worth-while dealing with the matter at some length, particularly as some important work by Stanworth (1953) which bears on this question is as yet unpublished.

Bretschneider and de Wit based their hypothesis mainly on work on the European bitterling *Rhodeus amarus*. Earlier work had indicated that ovipositor growth in bitterlings is stimulated by ovarian hormones secreted under the control of the pituitary (Tozawa, 1929 ; Artemov, 1936 ; Artemov & Smirnov, 1937), but the clear primitive light of this picture was later considerably clouded by the efforts of many workers to adapt the ovipositor-growth reaction as a pregnancy test (see Pickford & Atz, 1957). These workers usually added test substances to the water in which bitterlings were living, and their results showed that ovipositor growth could be induced by a wide variety of stimuli : androgens, oestrogens, progestins, corticosteroids, physical factors such as agitation or changes in light or temperature, and a wide range of chemical substances. Experimental ovipositor lengthening is quite definitely not a specific response to any particular hormone, substance or physical factor, and is most plausibly regarded as one manifestation of a generalized reaction to stress (van Koersveld, 1948, 1949 ; Stanworth, 1953). From what is known about the effects of stress in fishes, this view will explain the changes in the pituitary of the bitterling after immersion in solutions of steroid hor-

mones which, associated with extensive ovarian atresia, led Bretschneider & de Wit (1947) to postulate a specific pituitary factor causing atresia.

The general effects of stress in teleosts are now fairly clear. The most striking change is a pronounced hypertrophy of the adrenal cortical cells, which may start within two to six hours of exposure to cold, immersion in saline, injection of saline or intermedin, or implantations of optic lobe, head-kidney or opisthonephros (Rasquin, 1951 ; Rasquin & Atz, 1952 ; Chavin, 1956). These treatments do not produce adrenal hypertrophy in hypophysectomized goldfish (Chavin, 1956), indicating that the point of action of the stress stimuli is the pituitary or its superordinate nervous centres, an interpretation borne out by the pronounced adrenal hypertrophy induced by fish pituitary implants or mammalian ACTH injections in both intact and hypophysectomized fishes (Rasquin, 1951 ; Rasquin & Atz, 1952 ; Stanworth, 1953 ; Chavin, 1956). The adrenal consequences of shock are transitory, and the cells may show recovery to normality in a matter of four to six hours after exposure to stress (Rasquin, 1951 ; Rasquin & Atz, 1952).

Another marked result of stress in teleosts is seen in the mesoadenohypophysis. Two hours after cold shock treatment the basophils of this region in *Astyanax mexicanus* were vacuolated and their numbers reduced. No change was observed in acidophils (Rasquin, 1951). Similarly in the goldfish, immersion in saline set in train changes in these basophils, which became markedly reduced in size within six hours, were enlarged after twenty-four hours and returned to normal in three to five days ; again, the acidophils showed little or no change. Similar effects on the basophils followed injections or implantations of optic lobe, head-kidney, opisthonephros, saline or intermedin (Chavin, 1956). These same basophils are, on independent evidence (§ I F), the cells responsible for production of gonadotrophin and thyrotrophin, and Atz (1953) established that both thyrotrophs and gonadotrophs displayed marked cycles of degranulation and regranulation both at the beginning and end of a course of injections of saline, or cortisone, or ACTH. In goldfish, Chavin (1956) observed no changes in basophils after ACTH injections, but the acidophils became greatly enlarged and densely granulated, and cortisone caused the acidophils to increase in number and become heavily granulated, changes which suggest that these cells may be the source of endogenous ACTH.

The other symptoms in the stress reaction can be explained in terms of an initial release of ACTH from the pituitary causing hypersecretion of adrenal cortical hormones. In support of this interpretation, two hours after injection of saline or cold shock there is a massive extrusion of lymphocytes from the head-kidney and spleen. As in mammals, this appears to be a response to corticosteroids, since ACTH strongly and cortisone drastically produces this effect (Rasquin, 1951 ; Rasquin & Atz, 1952). Thyroid activity, judged histologically, is strongly depressed in stress situations. Like adrenal cortical hypertrophy, this did not occur in hypophysectomized fishes exposed to stress, nor did ACTH or adrenal cortical extract affect the thyroid in the absence of the pituitary, though potent in intact fish (Chavin, 1956), which suggests that stress affects the thyroid indirectly by way of a depression of pituitary thyro-

trophic activity by the elevated corticosteroid level, as in mammals (Money, 1955). Another, most important, consequence of injections of cortisone or ACTH in *Astyanax* was inhibition of oocyte growth and the conversion of many—not all—of the advanced oocytes into corpora atretica (Rasquin & Atz, 1952). In the stickleback, corpora atretica were formed in response to injections of oestrone, testosterone, saline and cod pituitary extract, or alterations in light and temperature conditions. In general, follicular atresia followed almost any environmental disturbance. All these changes also elicited adrenocortical hypertrophy accompanied by lymphocyte depletion in the head-kidney and spleen, indicating the enhanced production of corticosteroids (Stanworth, 1953). The pituitary was not examined, but it is reasonable to suppose that, as in *Astyanax* and the goldfish, the thyrotrophs and gonadotrophs were affected. In *Thalassoma bifasciatum*, sesame oil injections produced large-scale follicular atresia (Stoll, 1955).

The relevance of all this work to the hypotheses of Bretschneider & de Wit (1947) should now be clear. The changes in pituitary basophils and the formation of corpora atretica following the immersion of bitterlings in solutions of steroids did not signify cause and effect, as the Dutch workers concluded, but were typical reactions to stress conditions, both consequent on an increased endogenous production of corticosteroids. The accompanying ovipositor growth may have been partly in response to the administered steroids, since there is no reason to suppose that normal ovipositor growth is not under ovarian steroid control ; but in the experiments, ovipositor growth was so rapid, and occurred in response to so many diverse stimuli that it most probably was mainly a response to the internal changes in the stress reaction. Perhaps the suddenly elevated corticosteroids mimicked the normal action of the ovarian steroid hormone(s). It is relevant that peritoneal hypertrophy in trout, like ovipositor growth probably dependent on the ovary in normal circumstances, can be induced by injections of alcohol to the accompaniment of hypertrophy of the adrenal cortical cells (Ashby, 1957). The postulate of a special pituitary factor causing follicular atresia (Bretschneider & de Wit) has always seemed improbable, since a most effective way of inducing atresia is to remove the pituitary. Stanworth (1953) observed that when necrotic remnants of the oölemma remained in ovulated follicles the granulosa cells behave as they do in atresia, becoming hypertrophied and phagocytic, suggesting that in atresia the oocyte stops growing and becomes inert, and that in response to this the granulosa cells change their function, become phagocytic and form a corpus atreticum. Thus in spontaneous atresia, and in atresia induced experimentally by stress or by hypophysectomy, the primary event is inhibition of second phase oocyte growth. During this period of oocyte development, the thyroid and adrenal are both relatively hyperactive (§ II), and changes in the endocrine balance in stress—particularly a depression of thyroid function in association with high levels of the catabolic corticosteroids—would be expected to inhibit the intense anabolism involved in vitellogenesis. The fact that gonadotrophs, as well as thyrotrophs, are affected by stress conditions (Atz, 1953), indicates a transitory disturbance of gonadotrophin

output in association with the thyroid and adrenocortical changes, all of which would be expected to stop oocyte growth.

In follicular atresia, the granulosa cells hypertrophy and digest and phagocytose the oocyte, probably in response to changes within the oocyte consequent upon its ceasing to grow. The phagocytic behaviour of the granulosa cells is a property which they share with the cells of the coelomic epithelium from which they are ultimately derived, as Bragdon (1952) has pointed out. The persistence and organization of the lipid-charged granulosa cells into a corpus atreticum, or pre-ovulatory corpus luteum (Pl. 2, figs. 5 & 6), is another matter. It does not occur, or only to a slight extent, in those post-ovulatory follicles in which the residual oölemma has been phagocytosed (Stanworth, 1953) and may be a consequence of the relatively enormous amount of material ingested from the oocyte. The cells at this stage are heavily laden with sudanophilic material, which includes phospholipids (Stanworth, 1953), derived ultimately from the lipids of the digested oocyte (Pl. 2, fig. 7).

Later, the theca sends connective-tissue inpushings into the central mass of parenchymatous cells, together with blood vessels. This is the β-stage of Bretschneider & de Wit. There are indications, too, that some of the parenchymatous cells are derived from cells of the original theca layer, but there is no doubt that the granulosa makes the greater contribution towards the corpus atreticum. The structure later shrinks, presumably as material is removed via the bloodstream ; the lipid globules seem to run together to form extracellular pools, and the general picture is one of degeneration. A granular yellow pigment accumulates in the cells, and many cells migrate away into the stroma of the ovary (Bretschneider & de Wit, 1947 ; Stanworth, 1953 ; Ball, 1959). The final stage is described by Stanworth as " a small heap of cells full of yellowish granules together with fibroblasts and connective tissue fibres ". This is the γ-stage of Bretschneider & de Wit, and these bodies seem to persist in the ovary for long periods (Pl. 2, fig. 8). Sometimes, too, in older fish, small masses of healthy-looking β-stage cells can be found in association with blood capillaries (Pl. 1, fig. 4). In *Chloea sarchynnis*, in which the female develops a strong nuptial coloration during the breeding season, Kinoshita (1938) has described how the number of β- and γ-stage corpora atretica in the ovary varied, the spaces between follicles becoming largely filled by these bodies as the breeding season approached. After the breeding season, these cells disappeared and the inter-follicular spaces contained only connective tissue during the non-sexual period. He concluded that these bodies, which he regarded as masses of interstitial cells, secreted the hormone on which nuptial coloration depended. This coloration could be induced by steroid hormones, to which the fish were about twice as sensitive at the beginning of the breeding season as in the non-sexual period, presumably because of a higher level of endogenous ovarian steroids. Again, a pregnancy urine extract was highly effective in inducing nuptial coloration at the beginning of the breeding season, but had little or no effect during the quiescent season when few or no corpora atretica were present. These facts support Kinoshita's conclusion that the endogenous steroids originated from the corpora atretica.

Since follicular atresia affects oocytes throughout the second growth phase, as the ovary matures it comes to contain large numbers of β- and γ-stage corpora atretica, and the maximum number of these bodies occurs when, or just before, the ovary is fully developed (Bretschneider & de Wit, 1947 ; Stanworth, 1953 ; Ball, 1959). During the β-and earlier phases, the cells are negative in the Schultz test for cholesterol, but as the lipid runs together in globules at the γ-stage, it gives a strong positive Schultz reaction (Ball, 1959). It seems reasonable to infer from this that the cells of the earlier stages are actively utilizing cholesterol, the arrest of this activity consequent upon degeneration then allowing cholesterol to accumulate in an amount that can be detected by histochemical means. The activity of the cells, it is suggested, leads to the elaboration of ovarian steroid hormones. Cholesterol is a precursor of steroid hormones in mammals (Dorfman, 1957 ; Heard *et al.*, 1956 ; Chester Jones, 1957 ; Kritchevsky, 1958), and on general biological grounds we may assume this to be so, too, in fishes, with their similar steroids of ovarian (Dean & Chester Jones, 1959) and adrenal (Phillips & Mulrow, 1959 ; Chester Jones *et al.*, 1959) origin. The correlation between the number of corpora atretica and gonad maturation, the fact that they are universally present in fish ovaries (Buhler, 1902 ; Wallace, 1903 ; Fraser & Renton, 1940 ; Bretschneider & de Wit, 1947 ; Stanworth, 1953 ; Gokhale, 1953 ; Ball, 1959), their histological appearance, and the large amount of lipid and cholesterol evidenced during their regression, and—not least important—the absence of any other apparently secretory element in the fish ovary: all these support the idea that the atretic follicle is the source of the ovarian hormone(s). The morphological formation of the corpus atreticum, independently of pituitary stimulation, results from the response of the granulosa cells to the inhibited second-phase oocyte. In absorbing the oocyte, the granulosa cells will acquire lipids, including cholesterol which is present in considerable amounts in fish eggs (Needham, 1931). Whether the formation of steroid hormones in these cells from cholesterol is under pituitary control remains to be demonstrated ; such control is implied by the evidence of gonadotrophic control of secondary sexual characters in fishes and in other lower vertebrates with similar ovarian histology.

The derivation of interstitial endocrine cells in the ovary from atretic follicles is found also in birds (Marshall & Coombs, 1957) and mammals (Brambell, 1956 ; Simpson, 1959) though in both cases the cells are considered to derive ultimately from thecal cells, whereas in fishes, amphibians (Burns, 1932) and reptiles (Bragdon, 1952) the predominant cell of the corpus atreticum comes from the granulosa, though probably some thecal cells are incorporated. However, in some birds and mammals the granulosa cells appear to dominate in atresia (Brambell, 1956), and one author found that the interstitial cells in New World monkeys come from both granulosa and theca of atretic follicles (Dempsey, 1939). In view of the close similarity of their origin and of the appearance of granulosa and theca cells in their parenchymatous form—for example, in the mammalian corpus luteum (Brambell, 1956)—it may be that both types of cell are potentially capable of hypertrophy and a phagocytic response to dead tissue in the follicle, the emphasis having shifted in evolution

from the granulosa to the theca cell. In the vertebrates as a whole, then, either type of cell after participating in follicular atresia may take on an endocrine function in response to pituitary stimulation, though the phagocytic response in atresia, at least in the lower vertebrates, is independent of the pituitary and occurs after hypophysectomy. At first sight, this seems a remarkably uncertain way of obtaining steroid hormones, by utilizing cells and steroids accumulated during the destruction of a proportion of the developing eggs. But follicular atresia is exceedingly common throughout the vertebrates, and certainly in fishes every fully-developed ovary has its complement of corpora atretica. The experimental work on stress discussed earlier has shown how easily follicular development can be disturbed, leading to the production of corpora atretica. In this respect the ovary is so sensitive to environmental disturbances that it would not be surprising to find follicular atresia occurring so commonly in poikilotherms that the cells of the corpus atreticum, involved in the metabolism of cholesterol and other lipids, could come to be utilized as a source of steroid hormones. It would, indeed, be in line with current endocrinological thought to suppose that progesterone and perhaps oestrogens were produced at first simply as metabolic by-products in the removal of ovarian debris, and that certain structures and tissues subsequently acquired the ability to respond specifically to these substances in the blood stream. The fact that ovarian hormones seem to have profound metabolic effects on the liver and on fat deposition (§ III B) raises the possibility that one of the first functions of the newly-created hormone(s) might have been to maintain the general metabolic state necessary to allow the mobilization and deposition of large quantities of material in the developing eggs.* Other structures and behaviour patterns would be stimulated by the hormone(s) in approximate correlation with ovarian maturation, and might then come to be utilized as accessories and as secondary sexual characters in reproductive activities. It would then be advantageous, by way of ensuring closer correlation between gonad maturation and the secondary sexual characters, that the production of the hormone(s) should come to be stimulated by the pituitary gonadotrophins already responsible for the development and ovulation of the egg, so resulting in the establishment of the features typical of the endocrine regulation of reproduction in female vertebrates.

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* Injections of oestradial benzoate in goldfish appear to stimulate the formation of the phosphoprotein vitellin in the liver. This is probably transported to the ovaries and deposited in the developing oocytes, where it forms an important part of the yolk [Bailey, R. E., 1957, *J. exp. Zool.* **136**: 455].

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EXPLANATION OF THE PLATES

Plate 1.

Fig. 1.—Ovary of mature non-pregnant *Mollienesia latipinna*. β-stage corpus atreticum to the left, two fully developed oocytes, two first phase oocytes to the right. Smith's formol-dichromate, haematoxylin and eosin.

„ 2.—Ovary of adult *Mollienesia latipinna* 36 days after total hypophysectomy. Four days after the operation this fish gave birth to a normal brood. Some of the first-phase oocytes present during follicular gestation would normally then have grown to full size within a few days. In the absence of the pituitary this did not occur and the ovary contains only first-phase oocytes. Formol sublimate, haematoxylin and eosin.

„ 3.—Ovary of *Bathygobius soporator*. Bouin, haematoxylin and eosin.
Above : ovary of normal juvenile fish.
Below : ovary of juvenile fish one week after implantation of 3 adult pituitaries. [Reproduced from Stoll (1957), by permission of the New York Zoological Society].

„ 4.—Maturing ovary of *Coregonus clupeoides*. First and second phase oocytes. Two masses of β-stage cells derived from corpora atretica (arrowed). These resemble the cells shown in Fig. 6. Bouin, haematoxylin and eosin.

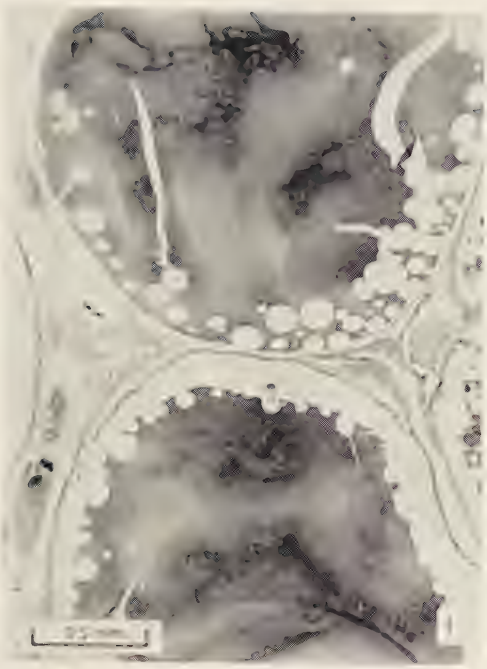
Plate 2.

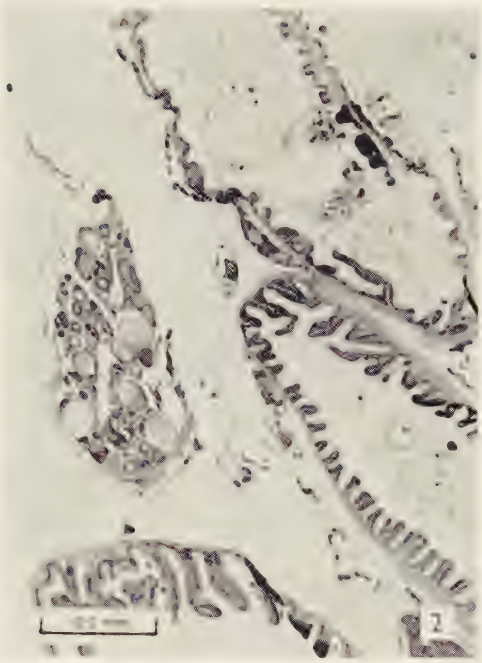
Fig. 5.—Corpus atreticum. β-stage, in the ovary of brown trout, *Salmo trutta*. Note capsule (theca) and the central mass of parenchymatous cells derived mainly from the granulosa. Small oocyte remnant arrowed. Zenker-formol, haematoxylin and eosin.

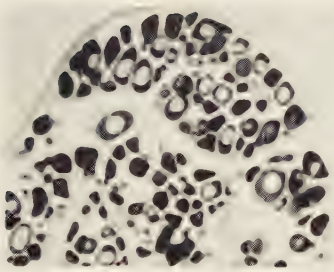
„ 6.—Part of corpus atreticum β-stage in brown trout ovary. Note resemblance of the parenchyma cells to the lutein cells of the mammalian corpus luteum. Bouin, Heidenhain's azan.

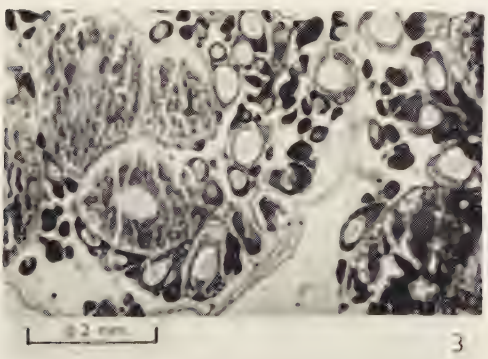
„ 7.—Corpus atreticum, β-stage, to show distribution of lipid mainly as intracellular droplets. Trout ovary. Formol-calcium, Sudan black.

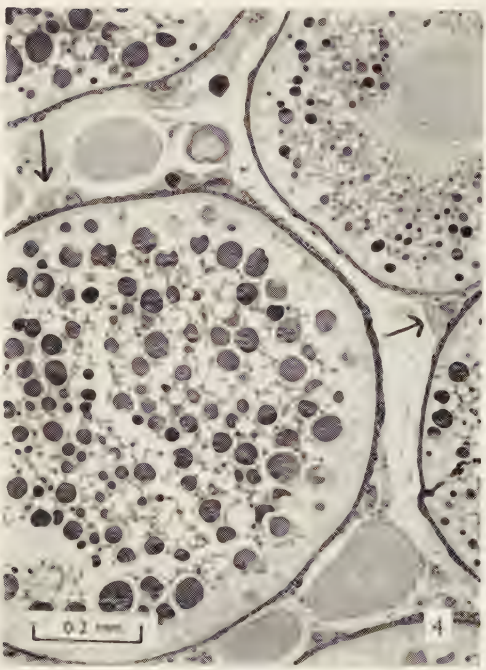
„ 8.—Corpus atreticum γ-stage, in trout ovary. The lipid at this stage runs together as extracellular pools or globules. Part of advanced oocyte at top of photograph. Formol-calcium, Sudan black.



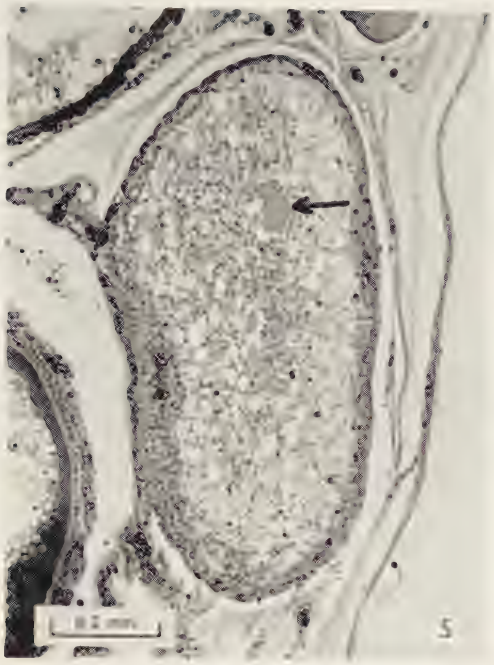


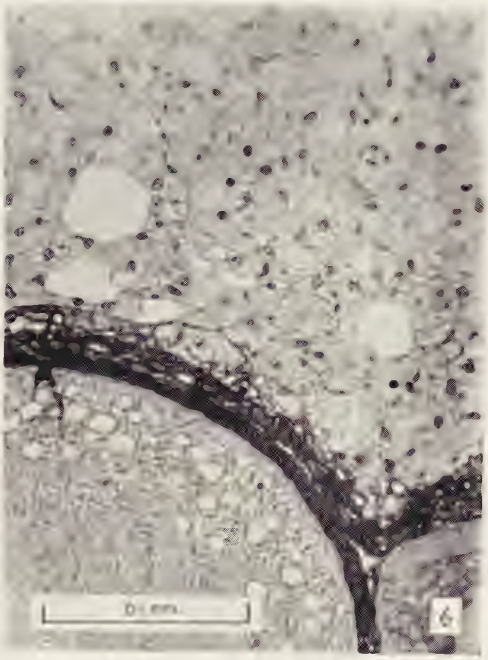


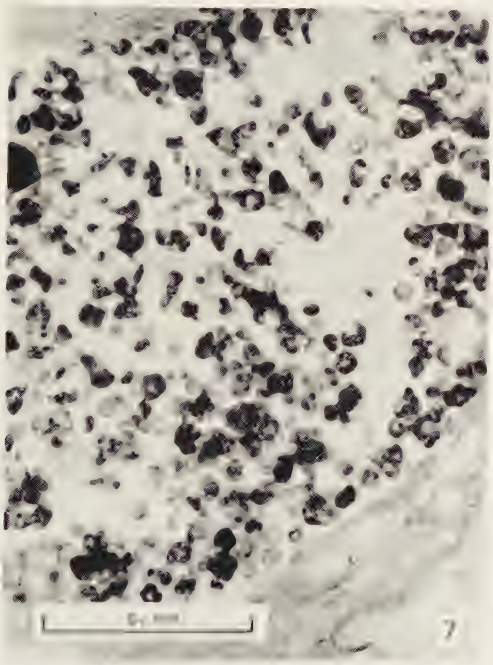


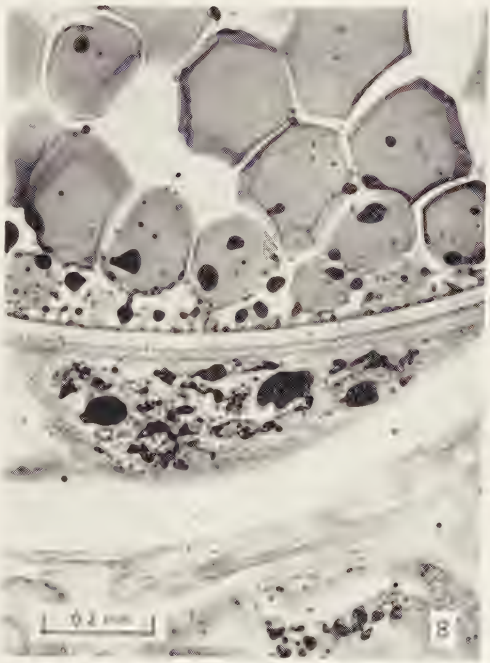


Reproduction in female bony fishes.









Reproduction in female bony fishes.

GENERAL DISCUSSION OF PAPER

Chairman : Professor I. Chester Jones

Mrs Woodhead : With reference to the problem of species-specificity of the fish pituitary hormones, I have recently carried out gonadotrophin assays of cod pituitary material which may be of interest. I injected cod pituitary material into immature female rats by standard techniques, and found that the fish pituitary did not cause an increase in ovarian weight, indeed in some assays the ovarian weight was significantly less than that of the controls. I then gave the cod pituitary material simultaneously with a standard dose of H.C.G. and found that the cod pituitary caused a significant reduction in the expected ovarian weight (as shown by controls receiving H.C.G. alone). The reduction in expected ovarian weight was proportional to the dose of cod pituitary given. Extracts of cod muscle did not have this effect, and therefore it did not seem that the fish material was toxic to the rats ; cod brain powder also had no inhibitory effect, and it seems that this " antigonadotrophic " effect was restricted to the cod pituitary. However, the cod pituitary did have a marked gonadotrophic effect when assayed upon fish (female roach).

REPRODUCTION IN MALE BONY FISH

BY

A. J. MARSHALL

Department of Biology, Monash University, Victoria, Australia

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INTRODUCTION

Before I was somewhat surprisingly invited to take part in this symposium it is almost true to say that my interest in fishes was confined to just a few individual species, notably, I think, trout, salmon and halibut. The trout, grilled ; the salmon, smoked ; and the halibut, baked. I suppose I should confess that I had paid some *small* attention to the reproductive endocrinology of one or two other species and, in fact, had come to the conclusion during the last few years that, if I were starting all over again, I would perhaps work mostly with fishes, and not birds, as my experimental material.

Actually, one of the things about modern bony fishes that most impresses me is how like birds many of them are. Perhaps, it may be thought, one should rephrase and say how much are birds like fishes. But it does not much matter how we express the idea. Although birds arose as late as in the Jurassic, there has been relatively little significant avian evolution since the early Tertiary (Simpson, 1946). It seems probable that all existing orders of birds had arisen by the Cretaceous, though it appears that the passerine radiation and success came later. Leaving out of account the relatively archaic chondrosteans and holosteans, we will see that the teleosts, too, arose as late as the Jurassic (e.g. *Leptolepis*). They became abundant (again like birds) in the Cretaceous. They expanded enormously in the Tertiary and have been radiating widely, and in many cases grotesquely, ever since. We will see how similarly, and often, these aquatic poikilotherms on the one hand, and volant homiotherms on the other, have converged in their patterns of reproductive behaviour. I refer, of course, to matters such as seasonal flocking, acquisition of territory, display of (sometimes seasonally acquired) sexual characters, the building of nests, " brooding ", and the exercise of parental protection and so on.

Pre-nuptial flocking is common in both birds and fishes. The flock is mutually protective, probably more certain in its path-finding and almost certainly self-stimulatory in relation to reproduction. Individuals sometimes assemble in millions (notable examples are shearwaters, *Puffinus*, and herrings,

Clupea). Norman (1951) cites evidence that freshwater roach (*Rutilus*) sometimes congregate in such numbers that the bigger fish push the small ones out of the water.

Many fishes, particularly marine forms, must do what no completely terrestrial vertebrate can do : they produce millions of meagrely yolked, buoyant eggs, of which many are fertilized, and a few produce an offspring that in turn survives to reproductive age. At the other end of the scale, however, are species of lake, river or sea-shore fishes that produce relatively few, often more heavily yolked, eggs which can be anchored or hidden, and are sometimes guarded as tenaciously as are those of birds.

The cyprinodonts in particular show many convergences with birds. In some the male is brilliantly coloured and indulges in an excitable display during the sexual season (see below). In species in which fertilization is internal the males periodically develop an intromittent organ. An additional specialization which prevents the dispersal of milt is the adhesive spermatophores in which the spermatozoa are impregnated and kept together. In a great number of fishes, of widely dissimilar modes of fertilization, sexual dimorphism is pronounced. Some species (e.g. carp, *Cyprinus*) seasonally develop nuptial tubercles which are lost after the spawning season just as wattled starlings (*Creatophora corunculata*) and one of the pelicans, *Pelecanus erythorhynchus*, develop and lose a fleshy head and a horny beak appendage respectively at comparable periods of the cycle. Among many (and particularly cyprinid) genera the males become more vivid during the breeding season. In its intensity the display of the Siamese fighting-fish (*Betta splendens*) is reminiscent of that of birds-of-paradise.

It is well known that the mere sight of another individual is conducive to ovulation in pigeons (Craig, 1908 ; Matthews, 1939). Much the same thing is now known to occur in fishes. Thus Aronson (1957) has shown that if an isolated female *Tilapia macrocephala* is allowed to see another individual (of either sex) in an adjacent aquarium the spawning frequency is increased by more than 100 per cent. Aronson cites comparable evidence obtained by Mosher with the sargassum fish (*Histio histio*).

Likewise, the sexually stimulatory effects of bird song is well established. Now comes evidence by Tavolga (1956) that the grunting noises of the male goby (*Bathygobius soporator*) probably similarly stimulate the female.

Since the development of elaborate hydrophonic apparatus during the last war, parts of the oceans have been found to resound with grunts, groans, rasps, squeaks and clicks. Many of these may subserve reproduction, as well as flocking functions. One is irresistably reminded of the whistling song of the sirens. Ichthyologists many years ago shattered our illusions by insisting that this has nothing to do with mermaids (nor even sirenians) and that the sounds are emitted by fishes of the genus *Sciaena*. It will be just one more illusion lost if we are next to be told that the song is not that of the sirens calling to the chaps, but in fact, the chaps calling up the sirens.

Numerous fishes, as well as birds, defend territories and attack savagely in their defence. More fishes than is generally realized build nests. Some lay,

and leave the eggs, but others guard them fiercely, sometimes aerating the water by tail movement. Nesting usually involves the shifting of gravel and stones, or the clearing of a space in vegetation. A great number of birds build this primitive kind of nest. Other fishes (e.g. certain sticklebacks) make rather more elaborate nests of seaweed or fresh water plants, sometimes gummed together with mucus in a manner reminiscent of the method employed by *Collocalia*, the edible-nest swiftlets.

The bitterling (*Rhodeus amarus*) is perhaps the most striking example of behavioural convergence of birds and fishes. This fresh-water cyprinid is adapted to a specialized breeding environment as irrevocably as is any hole-nesting bird. The male displays to the female in the vicinity of a freshwater lamellibranch, a territory which is said to exert on him a stimulatory effect perhaps comparable with that of the bower on bower-birds (Marshall, 1954). The female next lays in the mantle cavity of the mussel, after which the eggs are fertilized by the male. If a mussel is unavailable, reproduction is frustrated. The same almost always occurs when wild birds are deprived of their traditional breeding sites.

Much has been written in recent years about parental care in fishes (see particularly Aronson, 1957). Although no heat transference is involved, some species actually guard the eggs in a manner analogous to avian brooding. Again, a number of species (e.g. *Siphonostoma hippocampus*) carry their eggs in special structures which are even more elaborate than the special pedal device that has arisen in certain penguins (e.g. *Aptenodytes forsteri*). The astonishing buccal-brooding habits of many of the cichlids (*Tilapia*, and other genera) are too well known to merit detailed mention here. Even after the young swim free they dart rapidly back into the parental mouth in moments of danger.

I do not want to push the comparison between birds and fish too far. In any case, these behavioural convergences are not really very surprising after all. Both fish and fowl have basically the same neural and endocrine equipment ; and much the same ecological situations arose, and had to be overcome (and if possible exploited) whether the environment was wet or dry. Basic similarities in the reactions of many widely divergent animals—vertebrate and invertebrate, are emphasized by the work of Tinbergen (1951) and other modern ethologists.

Perhaps the most bizarre reproductive adaption of all is that developed by deep-sea ceratioid angler-fishes. It has been suggested that in the black depths the male might have difficulty in locating the female or might be eaten when it did. Whether such is the case of not, it is certainly true that one or more of the diminutive males *Edriolychnus schmidti* homograft themselves on to almost any part of the body of the relatively huge female. With a permanent vascular connection established, the males remain ectoparasitic for the rest of their lives. Each male is henceforth hardly more than a parcel of spermatozoa transported by an Amazon who, on dying, inevitably takes him with her. The precise means by which insemination occurs is still unknown.

THE HYPOTHALAMICO-PITUITARY AXIS

The remarkably comprehensive statement by Pickford & Atz (1957) makes it unnecessary for us to discuss this region here. They are at pains to reconcile the somewhat tangled terminology of previous authors and emphasize that, as far as has been determined, the hypophysial portal system, so well shown in tetrapods, does not occur. The question of the means by which hypothetical neurosecretory products are conveyed from the hypothalamic region is left open. Pickford and Atz cite evidence that "the meso- adenohypophysis [pars anterior, pars distalis or median zone] of teleosts appears to contain all the cell types that are associated with the mammalian pars anterior ". That the anterior pituitary exerts a profound gonadotrophic influence is certain, but little else seems to be indisputable. There is suggestive evidence that fishes produce prolactin.

Pickford and Atz tell us most of what is known about the reproductive physiology of fishes and, what is almost of equal importance, emphasize what is hypothetical or unknown.

ENDOCRINE ELEMENTS IN THE TESTIS

(a) *Interstitial cells and boundary cells*

No general agreement has yet been reached on the precise distribution and nature of the endocrine elements within the piscine testis. In the past, some workers have described an interstitium of aggregated Leydig cells of the typical vertebrate kind. Others have denied the existence of an interstitium. Reviews by Lipschutz (1924), Rasmussen (1928), Oslund (1928), Craig-Bennett (1931) and Hoar (1955) have dealt with the problem at varying length. It may be that work carried out in my laboratory can be helpful in settling the problem.

The male sex gland of the Craniata is, like other endocrine organs, extremely ancient in origin. It occurs in the Agnatha (for example *Petromyzon*) and throughout the gnathostome series with relatively little previously recorded variation except in the Urodela. With the above exception, and another described by Marshall & Lofts (1956), the interstitial gland is characteristically composed of groups of modified connective tissue cells which occupy the interstices between the seminiferous tubules.

These secretory Leydig cells have a generally similar appearance, and exhibit certain common histochemical properties in, for example, both jawless fishes and Man. The interstitial aggregation of Leydig cells may constitute either a permanent secretory unit (as in mammals) or it may be renewed at the end of each breeding rhythm as in seasonal birds (Marshall, 1949).

In the latter case, there is an annual development of large lipoidal cholesterol-positive Leydig cells from minute interstitial connective tissue cells. Unless Sudanophil colouring agents are employed, it is impossible to distinguish connective tissue cells from immature Leydig cells. This is generally responsible for the not-infrequent claim in the literature of breeding seasons that interstitial cells were absent at a stated period of the sexual cycle of some particular bird.

As mentioned above, Leydig cells have often been said to be absent in fishes. The reason for this may lie in the fact that two strikingly different endocrine arrangements occur in the testes of fishes. The first (Pl. 1, fig. 1) is the typical vertebrate interstitial gland (of Leydig cells) which is found in common teleosts such as the three-spined stickleback (*Gasterosteus aculeatus*) (Craig-Bennett, 1931), *Tilapia* spp., and the sprat (*Clupea sprattus*). This arrangement occurs also in lung-fishes and in the coelacanth *Latimeria* (as well as in the widely unrelated elasmobranchs *Scyliorhinus* and *Chimaera*). The second, and less-obvious arrangement (Pl. 1, fig. 2), arises not in the interstices but in the walls of the seminiferous tubules, and it occurs (for example) in the pike (*Esox lucius*), char (*Salvelinus willughbii*), and *Labeo victorianus*. Judged by published illustrations of Jones (1940) it probably occurs in the salmon (*Salmo salar*) as well.

The lobule, or crypt, wall of the teleost testis, like the walls of seminiferous tubules of tetrapods, is composed essentially of fibroblasts. In both pike and char these, in the absence of a secretory interstitium become seasonally modified into lipoidal, cholesterol-positive gland-cells cytologically similar to the interstitial cells of tetrapods. We provisionally named these boundary-cells.

In the pike at least, the annual boundary-cell cycle is essentially similar to that of the interstitial cycle in seasonal birds. After spermatozoa are shed, the residual contents of the lobules metamophose (Pl. 1, fig. 4) into a mass of cholesterol-positive Sudanophil material (Lofts & Marshall, 1957, and see below). The spent lobules are now bounded by unmodified fibroblasts. These grow, and many become lipoidal and cholesterol-positive at about the same time that the Sudanophil material within the lobules disappears.

It is almost certain that the lobule boundary-cells have the same endocrine function as interstitial Leydig cells. They finally expend their cholesterol at the pre-spawning period in April, after which the cycle begins anew. Most " conventional " histological techniques permit the loss of vital cytoplasmic lipids from boundary-cells and render them difficult to distinguish from ordinary fibroblasts. The use of Sudan dyes demonstrates their presence with unmistakable clarity provided they are in the process of maturation or are mature. It cannot be too strongly emphasized, however, that it is only during certain periods of the sexual cycle that the boundary cells are so clearly evident, and even then only when care is taken to preserve their fatty contents.

However distinct at first sight, the two types of endocrine cells are morphogenetically very similar. In most vertebrates, gonadotrophic influences operate on intertubular connective tissue and produce sexual function. In the exceptional fishes mentioned above, the influences are on peri-lobular connective tissue, apparently with a similar result.

In some tetrapod species possessing a typical interstitium of Leydig cells, odd tubule-wall fibroblasts sometimes manufacture lipids and assume a glandular appearance. Thus, in the anuran *Rana temporaria*, and in the reptile *Vipera berus*, a few tubule-wall fibroblasts may be modified in much the same way as occurs in pike and char. In these fishes, however, the phenomenon is constant. In them, the only interlobular elements seen by us

have been blood-vessels, lymph spaces and their contents and, during the first few weeks after spawning, what appear to be primary germ cells.

The constancy of a given glandular arrangement within the species perhaps unexpectedly provides a useful taxonomic tool. It would be interesting to discover whether the distribution of interstitial Leydig cells on one hand, and the boundary-cells on the other, falls into line with current teleost classification. Both char (family Salmonidae), which has lobule boundary-cells, and the sprat (family Clupeidae), which has Leydig cells, belong to the same order (Isospondyli) as at present constituted. Such a tool may be useful in determining the relationships of some of the groups often lumped together as the order Mesichthyes. It is obvious that this artificial assemblage is of polyphyletic origin. The presence of boundary cells on the one hand, or interstitial cells on the other, will not tell us where a given genus ought to be put. But it may emphasize where it should not.

It may be, too, that deeper phylogenetic issues are involved. The crossopterygian *Latimeria*, and the dipnoans *Neoceratodus* and *Protopterus* all possess a true interstitium. There seems little reason to doubt, therefore, that the tetrapods have inherited this " conventional " pattern through the crossopterygian, probably osteolepid, line. The Anura too, possess an interstitium. So, apparently, do all extant reptiles (*Sphenodon*, the Squamata, turtles and crocodilians) and birds (fifteen orders examined) on the one hand, and the mammals (Monotremata, Marsupialia and various eutherian orders) on the other (see Pl. 1, fig. 3).

Nevertheless, the position is otherwise in the Urodela.

Many years ago Champy (1913) and Aron (1924) described a boundary cell arrangement in European salamanders (*Triton* spp.) and Humphrey (1921) too, working with the North American proteid *Necturus* and plethodontid *Desmognathus*, described a similar glandular organization. More recently, Miller & Robbins (1954) have indisputably shown that common newts *Taricha* (=*Triturus*) possesses glandular boundary cells. Stieve (1920) failed to find such cells in the European *Proteus* and, according to Miller & Robbins (1954), suggested that Kolmer & Koppanyi (1923) had shown the presence of a " conventional " interstitium in the salamandrid *Pleurodeles*. Miller & Robbins (1954) examined the figures of Kolmer & Koppanyi and decided that they depict boundary cells. I have recently done the same myself and have reached the same conclusion.

The Gymnophiona too may be considered briefly. They are generally considered to be a degenerate stock of uncertain, but nevertheless amphibian, origin. The position in regard to the glandular arrangements of their testes is obscure. After a great deal of organization I succeeded in getting a small series of caecilians from the Seychelles but none of the males was in a sexual condition by which I was able to determine the nature of its secretory cells.

I am conscious that I have strayed from the theme of this symposium but I have done so with a purpose. One of the first things we argued about as senior students was the possibility of the diphyletic origin of tetrapods. In the emphatic duality of the glandular arrangements of the Anura on the one hand, and the Urodela on the other, it is possible that we may have evidence

of at least a more fundamental cleavage than that provided by divergence in highly adaptive characters such as limbs, lungs and the like. It is almost true that " the relentless modifying power of evolution does not spare a single structural element ", as Stresemann (1959) has said so well. But it is nevertheless difficult to see how environmental pressures could bring about a change from secretory activity in the interstitium to similar activity in cells of the tubule walls or what reproductive advantage could be achieved from such a switch.

One can see advantages in a change from a boundary cell arrangement to that of interstitial cells, which all tetrapod groups higher than Amphibia seem to possess. In terms of physiological efficiency, secretory units would be better located in the interstices of the tubules rather than within their walls. It is no accident that the centrally placed hypophysis, a mediator of external influences in both anterior and posterior lobes, has arisen where it is. It could not have arisen anywhere else. Likewise, one suspects that the male sex gland has arisen in the close neighbourhood of the tubules because its primitive function was concerned (along with hypophysial influences) with spermatogenesis. There is much evidence of the androgenic stimulation of gametogenetic development in several vertebrate groups, including fishes (references in Pickford & Atz, 1957). But once male sex hormones began to be utilized for a variety of other purposes (e.g. the seasonal influence of secondary sexual characters, accessory organs and behaviour), and the early vertebrates began to increase in size, it would be more economical for interstitial connective tissue, rather than tubule-wall fibroblasts, to subserve endocrine functions. There is in the interstitium room for a more massive secretory gland. There, too, its individual units can be more efficiently reached, and their products removed, via the immediately adjacent vascular system.

I do *not*, of course, claim that the seemingly constant occurrence of two apparently deep-seated glandular arrangements within the amphibian testis denotes a diphyletic origin from separate piscine stocks. However, I do throw the idea into the ring as a good way to start an argument among workers who are more learned than I in tetrapod phylogeny (See Addendum).

(b) *Tubule contents*

Many people have believed that the seminiferous tubules possess endocrine functions. Stieve (1921, 1923, 1926) went so far as to deny that the interstitium is secretory and to claim that the tubular elements alone are of endocrine significance. Today such views are of historical interest only.

Nevertheless, in recent years there has accumulated evidence that certain intra-tubular elements may possess an endocrine function. There is some suggestion that Sertoli cells may be oestrogenic in birds and mammals (Teilum, 1950 ; Siller, 1956). Sertoli cells occur in fishes (Craig-Bennett, 1931). A second possible endocrine element within the seminiferous tubules may be the mass of cholesterol-positive lipid material (Pl. 1, fig. 4) that arises rhythmically after the completion of spermatogenesis in birds (Marshall, 1949, 1955), reptiles (Marshall & Woolf, 1957) and at least some fishes (Lofts & Marshall, 1957). In mammals this material arises only after hypophysectomy (Coombs

& Marshall, 1956). In birds it has been shown by means of paper chromatography, and the Hooker-Forbes (1947) bio-assay method, probably to contain progestogens (Lofts & Marshall, 1959). We are now using the same methods to test for its presence in fishes and amphibians.

THE ENVIRONMENTAL CONTROL OF REPRODUCTION

Compared with the wealth of information available on the systematics, ethology and physiology of fishes, remarkably little concrete knowledge is to hand concerning how external factors regulate their reproduction. This is all the more surprising when it is considered how relatively easy it is experimentally to duplicate the natural environment of many small species.

There is plenty of evidence that bony fishes have adapted themselves to a variety of external influences that enable them variously to breed during all seasons of the year, and some species, throughout the year. The two principal external factors governing the gametogenesis in seasonal breeders appear to be light and temperature. In this respect bony fishes are again like birds. Because birds are homiothermous, and Rowan (1925, 1929) experimentally photostimulated juncos (*Junco hyemalis*) to reproductive activity in 84° of frost, almost all reviewers have tended to discount the importance of temperature in avian reproduction. Hardly anything could be further from the truth. Certainly *adult* birds are homiothermous, but their eggs and very delicate small young are not. And so, although gametogenesis can be stimulated to begin in very cold conditions, the sexual processes (especially those of the female) will halt abruptly under unusually cold conditions. The females in particular have evolved a sensitivity to external inhibitors and they do not expend their reproduction potential until conditions improve (Marshall, 1959.) Even the Megapodidae, which behave like some fishes and reptiles in burying and sometimes abandoning their newly laid eggs, must delay ovulation until rain falls and allows the production of damp heat in their incubators. They, as much as other birds, are controlled by the fact that no thermoregulatory device has ever arisen in an egg-shell. Thermo-regulation is achieved only as young birds mature. Furthermore, at least one species of megapode (*Leipoa ocellata*) regulates the temperature of the incubator after it lays (Frith, 1953).

Although the seasonal temperature range in water is not as great as ashore, it would be predicted that, in temperate zones, water temperature would be found to be an important regulator of the reproductive cycles of cold-blood animals like fishes. Various species of fishes (Turner, 1919 ; Craig-Bennett, 1931 ; Lofts & Marshall, 1957) and at least one reptile (Volsoe, 1944 ; Marshall & Wolff, 1957) take advantage of the high temperature of summer to restart their gametogenetic processes almost immediately after the end of the breeding season. There follows a period of inactivity, or at least greatly reduced activity, during the subsequent coldest months. Craig-Bennett (1931), working with the three-spined stickleback (*Gasterosteus aculeatus*) concluded that temperature was the principal regulatory factor and, despite Rowan's recent demonstration with birds, that photoperiodic fluctuations were unimportant. However, Hoover (1937), Hoover & Hubbard (1937),

Bullough (1939) and Van den Eeckhoudt (1947) were soon to reveal that in brook trout (*Savelinus fontinalis*), minnow (*Phoxinus laevis*) and stickleback (*Gasterosteus aculeatus*) at least, photoperiodicity was, in fact, exceedingly important.

I often think that we might almost use the symbols " B.R." and "A.R." in this field of study. I mean, of course, " Before Rowan " and "After Rowan ". The fish studies that gave evidence of photo-periodic control were conducted after Rowan's initial work and in part the results might have been predicted. What could *not* have been predicted were the results of the ingenious experiments of Hoover (1937) and Hoover & Hubbard (1937). They, long before Bissonnette's (1941) work with goats, showed that a vertebrate animal could somehow adjust its sexual reaction to decreasing day-lengths. This was a remarkable advance ; and their work has been substantiated to the degree that their discovery is now used in commercial trout fisheries (see Hazard & Eddy, 1951 ; Corson, 1955). Later, Yeates (1949) showed that the cycles of Southdown domestic sheep are susceptible to decreasing day-lengths. So may be the cycles of birds that begin their gametogenesis in autumn. However, so far only one species has been tested and the results were negative (Marshall & Serventy, 1959).

Harrington (1950), with the cyprinid bridled shiner (*Notropis bifrenatus*), and Baggerman (1957) with the three-spined stickleback, have produced unequivocal evidence of dual control by heat and light. Baggerman's study is probably the most convincing of its kind yet published. It is additionally important in its conclusions regarding migration and the influences, and osmotic implications, of thermo-controlled thyroid activity (see also Hoar, 1939, 1957, 1958 ; Barrington & Matty, 1954). It has sometimes been suggested that in birds as well as fish, migration begins in the absence of gonad development. In *birds* these conclusions are due to the use of inappropriate techniques. Further, as Witschi (1945) showed, changes in the accessory sexual apparatus can occur as the result of an extremely small amount of sex hormone and at a time when histological evidence in the gonads is barely visible. In the pike (*Esox lucius*) the correlation between boundary cell activity and movement to the spawning grounds is clear-cut and unequivocal (Lofts & Marshall, 1957).

Frost (1951) has shown that in Lake Windermere in England two well-marked populations of the salmonid char (*Savelinus willughbii*) occur and breed at different times of the year. One group spawns in the autumn and the other during the spring. Again, Määr (1948) (cited by Fabricius, 1951) has evidence that different populations of whitefish (*Coregonus*) in Swedish Lapland spawn at different times and at different depths. Here the reproduction seems to be correlated with temperature.

We have so far considered only temperature and light as external stimuli. Among birds a multiplicity of other factors (safe nesting site, availability of nest materials, food supply, behavioural reactions, etc.) significantly influence the internal rhythm of reproduction and the culmination of the sexual season (Marshall, 1959). In certain species of fish (e.g. bitterling) some of these factors are as important as they are in birds. In the species of bony fishes

that broadcast their gametes such factors may be unimportant. After all, if the temperature and daylength are adequate to stimulate the fishes' reproductive cycle, it is probable that those very factors are also producing the food on which the resultant young will be fed. And it is the abundant presence of food of a kind suitable for the newly emergent young that is one of the primary factors in successful reproduction and the survival of the line.

The question of reproduction cycles in equatorial regions inevitably arises. In an environment in which temperature and photoperiodic changes are negligible throughout the year, do fishes breed all the year round, or are their cycles " timed " by local external factors ? Data are scanty, but Lowe (1955, 1956) has evidence that some species of the cichlid *Tilapia* can breed at any time of the year in natural conditions in East African lakes. The same is almost certainly true of some species of *Haplochromis* (Greenwood, unpublished) but it is not known how often a given individual reproduces under natural conditions.

Certain other East African lake fishes (e.g. the catfish *Clarius mossambicus*, the mormyrid *Marcusenius nigricans*, various species of *Barbus* and the cyprinid *Labeo victorianus*) have rigidly controlled breeding times. Greenwood (1955) has described how, after heavy seasonal rainfall, *C. mossambicus* congregates at dusk and moves up lakeside streams during the dark. Before the rains these were dry or contained only a trickle of water. Thousands of fishes of both sexes reach their traditional, now flooded, spawning grounds beyond the papyrus fringing the lake. Spawning occurs. Most of the adults leave the area by dawn and all have gone by the following mid-day.

The length of the period during which the breeding ground will remain available depends on the extent of the rainfall. The developmental rate of *C. mossambicus* is possibly correlated with this hazard. The embryo is at first anchored by means of an adhesive disk to dead vegetation and other debris on the flooded banks of the stream. It becomes a free-moving larva a mere twenty-four to thirty hours from the time of fertilization and tends to move into the main stream even though it is still dependent on flooded vegetation. By the time the stream subsides the young have followed their parents as far as the lakeside. If the rains do not persist and the stream subsides rapidly there is great juvenile mortality though the parents, of course, have escaped.

It is of great interest that the growing young ascend the stream during each subsequent flooding. They make " dummy runs ", just as do young shearwaters (*Puffinus*) of migratory species before they themselves reach a reproductive age. Greenwood and I are at present working on *C. mossambicus* in an attempt to elucidate the external and internal factors involved in its breeding migration.

Undoubtedly, we think, the lake-locked *Clarias mossambicus* populations are descended from riverine stock and it seems that their peculiar breeding migrations are probably to be looked upon as the retention of ancestral habits. It will be recalled that the sea lampreys (*Petromyzon marinus*) which have adapted themselves to a lake-locked existence in Lakes Huron, Michigan and Superior of North America, still assemble near the mouths of streams and,

when sexually mature, make their temperature-stimulated migratory runs upstream to spawn (Applegate, 1947). The colonization of these lakes (and the exploitation of the resident trout and other fishes) is extremely recent : the first lampreys were found in Lake Erie as late as 1921.

The African lungfish *Protopterus aethiopicus* is another seasonal breeder (Greenwood, 1958). It, however, does not ascend rivers but builds a lakeside nest (which it guards savagely) during the wet season. The young, in contrast to those of *C. mossambicus*, take about fifty-five days from the time of fertilization to the resorption of the yolk. They leave the nest a little later.

I will conclude with a few brief remarks on the phenomena known in *Tilapia* and other species as " runting ". As everybody knows, various species of *Tilapia* (particularly *T. mossambica*) have been introduced into many protein-poor parts of the world for culture as food fishes. Unfortunately, when these species are transferred from their natural environment to shallow artificial canals, ponds or dams their growth-rate is often depressed (they " runt ") and they begin to breed when a few inches long. The ponds become populated by such runts and the object of acclimatization is defeated. Beauchamp (1953) made the very plausible suggestion that when *Tilapia* is taken from its relatively dim natural environment to shallow tropical ponds the added illumination may lead to the stimulation of the anterior lobe with a resultant premature reproduction, retardation of growth and " runting ".

When I was guest scientist in the E.A.F.R.O. at Jinja a few years ago I began a simple experiment to test this idea and I expect that the results, positive or otherwise, are now available. Certainly we know that the cycle of at least one equatorial bird, the multitudinous weaver-finch *Quelea quelea*, can be experimentally photostimulated, even though photoperiodicity is without influence on it under natural conditions (Marshall & Disney, 1956, 1957).

SUMMARY

1. The remarkable convergence in reproduction behaviour between birds and certain groups of bony fishes is discussed.

2. In the testes of Osteichthyes two distinct glandular arrangements occur (a) *interstitial Leydig cells*, which arise from inter-tubular connective tissue ; and (b) *lobule boundary cells*, which arise from fibroblasts surrounding the seminiferous tubules.

3. The interstitial arrangement typical of vertebrates is found in *Petromyzon*, many bony fishes (including *Latimeria* and the Dipnoi), in elasmobranchs, and in the Anura, Reptilia, Aves and Mammalia. The boundary cell arrangement has been demonstrated to occur in certain bony fishes, and will be found in many more when it is specifically looked for. The occurrence or otherwise of one of these different glandular arrangements may provide a useful tool in fish taxonomy.

4. Although the Anura possess interstitial cells, the Urodela (as far as is known) have boundary cells. Purely speculative remarks are made concerning possible phylogenetic implications of these phenomena.

5. Bony fishes, like birds, have adapted themselves to a variety of external factors in the regulation of their reproduction cycles. Some equatorial species breed all the year round but the cycles of others are rigidly controlled.

6. The phenomenon known as " runting " is mentioned briefly.

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EXPLANATION OF THE PLATE

Fig. 1.—Typical " vertebrate " interstitium of lipoidal Leydig cells in the African lung-fish *Protopterus aethiopicus* under high power (×490). (Formal calcium and Sudan black at 10).

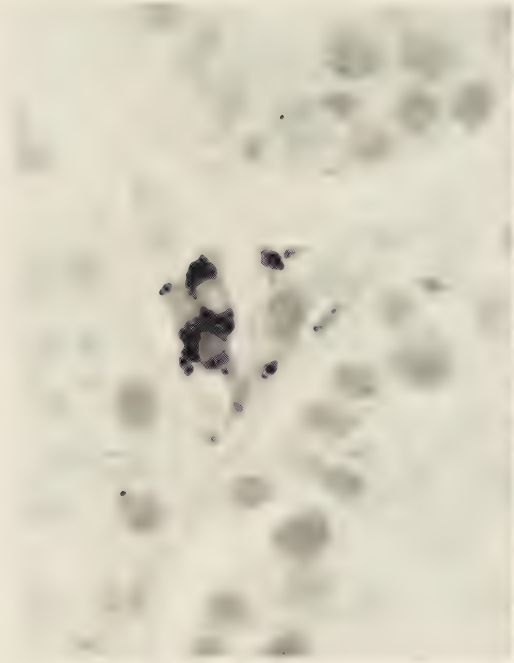
„ 2.—Lipoidal boundary cells lining the seminiferous lobules in the British char *Savellinus willughbii* under low power (×76). (Technique as in Fig. 1.)

„ 3.—Unusual arrangement of lipoidal interstitial Leydig cells as seen in *Chamæleo* (sp?). In this reptile there is a superficial appearance of boundary cells which is caused by the extension of the secretory interstitium right round many of the seminiferous tubules (×490). (Technique as in Fig. 1.)

„ 4.—Post-nuptial tubule lipids in the Australian lung-fish *Neoceratodus forsteri*. This material arises cyclically as part of the internal rhythm of reproduction (×76). (Technique as in Fig. 1.)

ADDENDUM

In a private conversation Professor D'Ancona has kindly volunteered extremely interesting information concerning the ontogeny of the two secretory tissues discussed on p. 143. He has pointed out that in amphibians (Witschi,



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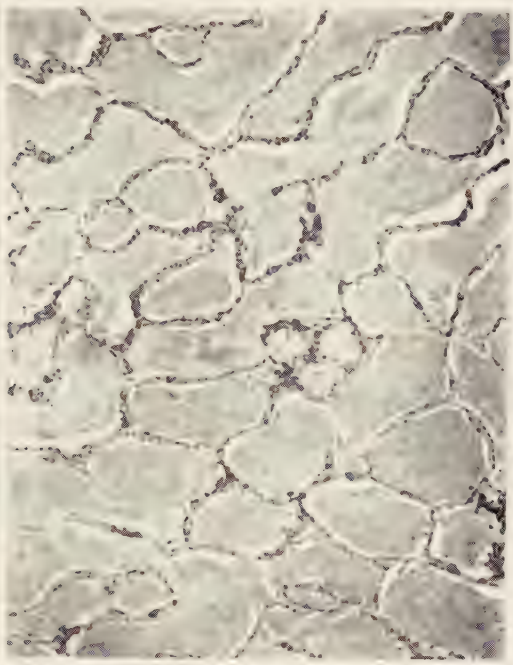

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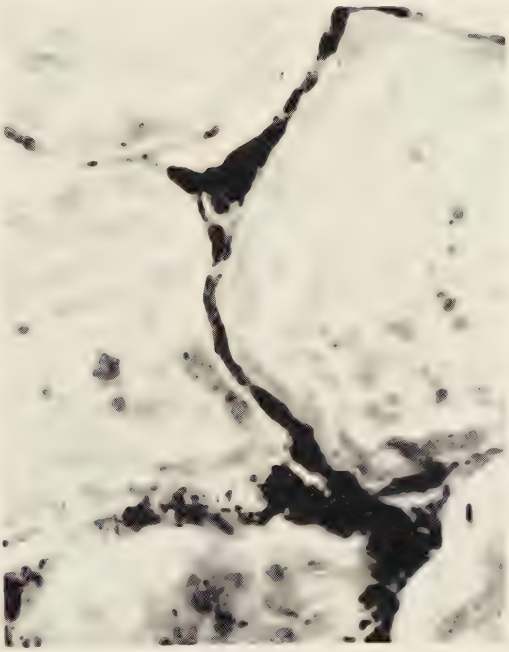

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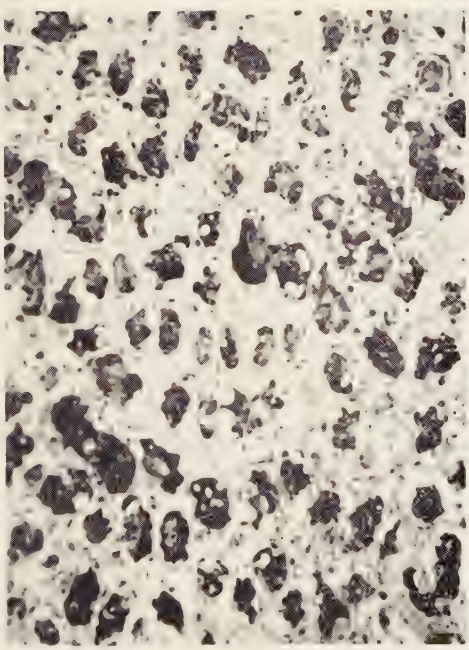

4

Reproduction in male bony fish.

1956) as well as in Amniota (Witschi, 1956 ; Vannini, 1950), and selachians (Chieffi, 1952) the gonad somatic substratum in general originates from two different embryogenetic sources which come to form the medulla and the cortex. In teleostomes, on the other hand, there is no part corresponding to the medulla ; therefore the whole somatic substratum of the gonad corresponds to the cortex of other vertebrates (D'Ancona, 1956 ; Ashby, 1952). " It can be assumed that in the latter the incretory cells of the male gonad derive from the medulla, having a common origin with the interrenal blastema (Vannini & Sabbadin, 1954), whereas in teleosteans (when incretory cells are present) they are formed in the substratum derived from the peritoneal wall."

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VIVIPARITY IN FISHES

BY

E. C. AMOROSO, F.R.S.

*Department of Physiology, Royal Veterinary College, London, N.W.*1

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VIVIPARITY IN FISHES

The study of the dependence of the vertebrate embryo upon the environment and the many devices for respiration and for the absorption of such nutrients as are present in the fluids bathing the developing egg, are so complex that to review them in a short paper is impossible ; and in any case, the excellent discussion by Matthews (1955) makes a further attempt at a comprehensive treatment unnecessary. Nevertheless, the present occasion, concerned as it is with " Fishes " provides an opportunity to consider in some detail two small groups, the Selachii and the Teleostei. Though limited in scope, this approach will serve to illustrate the nature of the problem in two kinds of evolutionary systems and to build a picture which may be filled in or erased by discussants and readers.

Retention of offspring on or within the body of the parent as a means of providing protection and nutrition is widespread among animals, many variants having arisen independently many times in widely separated taxonomic groups. A study of the literature reveals, moreover, that there has been a certain degree of parallelism in the evolution of the adaptations, but that there also is a surprising amount of diversity in the adaptation of fishes, including super-foetation and, in some extreme cases (e.g. Embiotocidae) retention of young within the body of the parent until they are sexually mature.

The many mechanisms for holding eggs at the surface or associating them with photosynthesizing plants found in *Osteichthyes* demonstrate that for development the oxygen and water requirements are difficult to reconcile in fresh water. Much of the transporting of eggs and pre-natal care in fish seems evolved to ventilate them. To protect them further from anaerobic situations, eggs and young may be carried in various cavities of the body, of which the oviduct is only one.

External appendages (e.g. *Kurtus gulliveri*, *Aspredo laevis*), mouth cavities (e.g. *Arius falcarius*) and specialized pouches in the male (e.g. *Hippocampus antiquorum*, *Syngnathus typhle*), the ovarian follicle and ovarian cavity or even the respiratory system of an un-related animal (e.g. *Rhodeus amarus*), have all been utilized as sites for embryo retention.

That male as well as female structures may provide nutrient fluids suggests that in mammalian evolution the functioning of the mammae has only recently become confined to the female, explaining their slight sexual dimorphism.

OVIPARITY, OVOVIVIPARITY AND VIVIPARITY

Amongst fishes, there can be found all gradations from " oviparity ", in which eggs are not retained in the body after maturation and fertilization, through " ovoviviparity " to " viviparity ", in both of which the eggs are retained within the body of the parent and the young are born alive. This latter distinction is based on the difference between live bearing forms which retain an egg with enough yolk to carry the embryo through its development and those in which there is an intimate nutritive connection between mother and offspring. The latter are considered truly viviparous. In the present context, however, this terminology is too rigid and bears the implication that each condition is static and that all cases must fall within one or another of these categories. On the contrary, there can be no sharp line of distinction among the different types since there are many instances of live bearing, in teleost fishes particularly, which cannot be classified strictly as ovoviviparous nor as viviparous, but rather as ovoviviparous with a superimposed viviparity (Turner, 1933). Thus, in the family Goodeidae, where the first stage of development takes place within the ovarian follicle, the interval between fertilization and the release of the young embryos into the ovarian cavity (technically interpreted as " hatching ") is relatively brief. The young individual, while in the follicle, relies only upon its small amount of yolk, most of which is absorbed. Thus, development is first ovoviviparous.

After the embryo is hatched, it enters the second stage, during which it lies within the ovarian cavity and depends only upon the ovary for nutrition.

This second stage is greatly prolonged and is concluded when the embryo is born. In this case " hatching " and " birth " are separated by a relatively long time and development is genuinely viviparous.

On the other hand, some cyprinodonts seem to be just on the borderline between oviparity and ovoviviparity, for Amemiya & Murayama (1931) noted that the eggs of *Oryzias latipes*, the Japanese medaka which normally develop outside the parent's body can sometimes do so within it. Unfortunately, few biochemical investigations have yet been made on the kind and extent of interchange between offspring and parent among teleost fishes, and so the extent of physiological dependence of the embryo upon the mother is not surely known. (*See*, however, p. 172 and also Konopacki & Erecinski, 1932 ; Leiner, 1936, 1937, and Needham, 1942.)

THE EVOLUTION OF VIVIPARITY IN VERTEBRATES

During the transition from oviparity to viviparity in fishes, reptiles and mammals, profound changes have occurred in the nature of the egg and in the structure of the female animal (Amoroso, 1955 a, b, c). These evolutionary innovations have involved : (1) a decrease in the number of eggs which are shed ; (2) perfection of internal fertilization ; (3) the utilization of the yolk sac for absorbing secretions and other nutrients ; (4) intra-uterine care of a few young to relatively advanced stages in development ; (5) the participation of endocrine glands in extending gestation, with the object of retaining eggs in the genital tract for periods beyond the limits of the normal sex cycle ; and (6) the adaptation of the endocrine control of lactation in preparation for early nutrition of the new-born.

The most primitive type of development is probably that in which innumerable sperm and innumerable minute, naked eggs, poor in yolk were shed into the surrounding water, where fertilization was effected without association of the parents themselves. However, with the appearance of land forms certain new requirements to ensure survival became necessary. The first of these was for some loose association of the sexes, which may have begun as the result of chemical stimulation due to substances extruded at spawning time. Another was for some sort of protection against dessication.

As well as the secretion of a protective envelope around the egg, developments for assuring greater survival have included increased storage of nutritive material in the form of yolk, and a concomitant reduction in the number of eggs produced. Moreover, the production of a large cleiodoic egg would, in general, presuppose a parallel development of sexual association to ensure internal fertilization before the egg was completely closed off from its environment.

With the development of these innovations an early free-living aquatic existence had now become impossible and a more protracted developmental period was only possible if there were sufficient reserve food materials and if the larval stages could be compressed into the embryonic period. But the larger and less numerous the eggs became, the more valuable they were to the species and hence we see arising various kinds of arrangements for the care of developing embryos. Such close contacts led ultimately to the

complete retention of the eggs within the parent organism, at some level in the gastro-intestinal or reproductive tract, or in some specially prepared marsupium or pouch. Such embryos were provided with ample supplies of yolk, and obtained little, if any, additional food from the parent.

In some such way ovoviviparity arose, but from this point onwards evolutionary changes in reproduction have involved a loss of yolk concommitant with specialization of the embryonic membranes and maternal tissues as an organ of nutrition and internal secretion (Amoroso, 1955 b). The selachian and teleost fishes offer special opportunities for the study of these evolutionary changes, for they include oviparous, ovoviviparous and purely viviparous forms.

In vivipara the utilization of the yolk-sac and the chorio-viteline circulation for the absorption and transport of uterine milk, and other nutrients, seems to have been the first method used to supplement a reduced yolk supply (Amoroso, 1952). Some elasmobranchs and most marsupials make use of such a placental connection. The marsupial *Perameles*, many reptiles, and all the eutherian mammals have a chorio-allantoic placenta as well, and the latter supplements the functions of the yolk-sac placenta. In this connection it must also be observed that in the ovoviviparous elasmobranchs and teleosts the uterine wall and the ovaries respectively, already play an important role in providing embryotrophe for the young individual after the yolk has been used up, and this may be supplemented by material derived from the autolysis of non-viable eggs and embryos (Amoroso, 1955 a).

THE SIGNIFICANCE OF THE CORPUS LUTEUM IN THE EVOLUTION OF VIVIPARITY IN VERTEBRATES

Proceeding and associated with these evolutionary changes was the development of corpora lutea. In non-mammalian vertebrates, however, these luteal structures are not invariably associated with viviparity. Indeed, they have been described in oviparous and ovoviviparous selachians (Wallace, 1903 ; ten Cate Hoedemaker, 1933 ; Hisaw & Abramowitz, 1938, 1939 ; Hisaw & Albert, 1947 ; Bretschneider & de Wit, 1947), as well as in ovoviviparous and viviparous teleosts (Bailey, 1933 ; Mendoza, 1943 ; Bretschneider & de Wit, 1947) amphibia (Lamotte & Rey, 1954 ; Amoroso, 1955 d ; Gallien, 1959) and reptiles (Weekes, 1934, 1935 ; Rahn, 1938 ; Boyd, 1940, 1942 ; Miller, 1948 ; Kasturirangen, 1951 ; Bragdon, 1951, 1952, 1953 ; Bragdon *et al.*, 1954 ; Amoroso, 1955 d ; Matthews, 1955 ; Panigel, 1956) and in mammals (Asdell, 1928 ; Harrison, 1948 ; Brambell, 1956 ; Hisaw, 1959), where they undergo their greatest specialization, they first appear in the oviparous monotremes. They are associated in vivipara with the retention of the embryos and in ovipara with the storage of eggs in the oviduct.

Te Winkel (1950) suggested that ovarian hormones present at the time of ovulation or slightly preceding it in selachii, may stimulate the secretion of the egg case by the oviducal gland. Moreover, Matthews (1955) believes that luteal hormones may stimulate the hypertrophy of the uterine mucosa so that the folds wrapping the eggs in separate compartments are formed in

Mustelus and others, as well as the more elaborate yolk-sac placenta in *Scolidon* (see below).

In the viviparous blenny, *Zoarces viviparus*, Bretschneider & de Wit (1947) state that the granulosa and theca interna of the discharged follicle are transformed into corpora lutea. In addition to producing a copious secretion of embryotrophe for the nourishment of the young, these bodies, through their secretions, may participate in the maintenance of pregnancy since they do not involute until some time after parturition. At the same time large numbers of developing but immature follicles are transformed without ovulation into corpora lutea which remain a conspicuous feature of the ovary throughout the whole of pregnancy and may, likewise, contribute to its maintenance.

In *Neotoca bilineata* described by Mendoza (1938, 1943) the evacuated follicle does not assume the " windswept " appearance of the follicle of *Xiphophorus* (Bailey, 1933), nor does it become hypertrophied as in *Sebastes murinus* (Williamson, 1910) and *Anableps anableps* (Turner, 1938 a). In *Neotoca* there is no evidence of pronounced physiological activity ; actually the follicle cells are small and shrunken (Mendoza, 1943).

Finally, in many species of the four families of Cyprinodontes, fertilization is accomplished within the ovarian follicle and in some, but not all, the embryos are retained in the follicle until just before parturition. Accordingly, fertilization within the follicle prevents true ovulation, and, since ovulation is believed to be controlled by a pituitary hormone, it would appear that some endocrine change has been brought about by, or is concurrent with, the new development of intra-follicular fertilization. That such a change is integrated into the teleostean endocrine cycle of reproduction seems a permissible deduction.

One of the problems confronting mammals in the evolution of viviparity was that of extending gestation with the object of retaining eggs in the genital tract for periods outlasting the duration of the normal sex cycle. In the monotreme mammals the situation is similar to that of other viviparous vertebrates in which internal fertilization occurs and merely involves the retention of eggs until the shell is formed. Likewise, in the marsupial *Didelphys virginiana*, in which pregnancy lasts about twelve days and in which the luteal phase of the cycle is so prolonged as to be comparable with true pregnancy in duration (complete pseudopregnancy), parturition is correlated with involution of the corpora lutea. Since the ovaries are necessary throughout gestation in this species (Hartman, 1925), it is assumed that the maintenance of pregnancy does not involve hormonal mechanisms other than those that participate in the regulation of the oestrous cycle. In higher mammals, on the other hand, as gestation is prolonged other and more complex controlling mechanisms appear. The evidence points to the placenta as being implicated in these changes, the direction of which is towards the endocrinological specialization of this organ as an adjunct to the ovary and to the pituitary (Amoroso, 1952 ; 1955 b, c, d).

SELACHIAN OVIPARITY AND OVOVIVIPARITY

The elasmobranch fishes, containing as they do purely oviparous forms (such as the dogfish, *Scylliorhinus canicula*), ovoviviparous forms (such as

Mustelus vulgaris and *Trygon violacea*) showing all grades of relationship between the embryo and the maternal uterus, as well as truly viviparous forms (such as *Mustelus laevis*) in which there is a yolk-sac placenta, offer special opportunities for the study of evolutionary innovations that emphasize the dependence of the embryo upon the environment.

In oviparous forms, the eggs are large (megalecithal) and correspondingly reduced in numbers. When released from the ovarian follicle the egg enters the oviduct (a derivative of the segmental duct) where it is fertilized and acquires a horny case. Fertilization is effected before the horny case is secreted by the oviduct, intromission being accomplished through the clasper of the male during copulation. But, internal fertilization does not necessarily occur because the egg is encased in a horny shell, for the egg of Myxine, similarly enclosed in a shell, is fertilized externally (Matthews, 1955).

On the other hand, internal fertilization is a necessity in truly viviparous forms such as *Mustelus laevis*, since the eggs, enclosed in their comparatively thin membranous shells, are retained in the lower part of the oviduct until development is completed. In this species the embryos are separated in compartments and there is a specially modified condition of the yolk-sac so that it burrows into the maternal tissues (see below).

Gastrulation and embryo formation in selachians are notable for their adaptation to the large size of the eggs. In most species the yolk mass, to which the early embryo is attached, is comparatively enormous, the proportions gradually becoming reversed as development proceeds. At the time when embryo formation enters the primitive streak stage, a mesodermal layer begins to grow in from the lip of the yolk blastopore. Consequently, the yolk-sac early becomes three layered, which is important in connection with the development of the circulatory system and in the absorption of nutrients from the environment.

DEPENDENCE OF SELACHIAN EGGS ON THE ENVIRONMENT IN OVIPARITY, OVOVIVIPARITY AND VIVIPARITY

Ranzi (1929 ; 1930 a, b ; 1932 a, b, c) was the first to show that the development of oviparous forms such as the dogfish (*Scylliorhinus canicula*) exactly resembles that of other marine eggs, such as the squid egg (*Sepia officinalis*) in obtaining 60 per cent of inorganic substance which it requires, and about 70 per cent of the water from the acquatic environment and not from the maternal organisms (Fig. 1 and Table 1). This striking intake of water and inorganic material from the environment is doubtless aided by strong ciliary currents within the perivitelline space of the cephalopod egg, and in *Scylliorhinus* by the circulation of water which is maintained from a very early age through the unsealed end of the egg case by the constant rhythmic movements of the embryo (Matthews, 1955).

Among the elasmobranchs, numerous examples of the birth of living young exist in which the embryo within the uterus is still nourished by the yolk in the yolk sac (Popovici, 1938). There are, however, considerable variations in the composition of the uterine fluids bathing the egg cases while the embryo develops (Table 2), indicating that in these ovoviviparous forms the uterine

wall already plays an important part in providing food material, for the young individual, by its glandular activity (Ranzi, 1934). Thus, in *Torpedo* it is abundant but dilute (Reach & Widakowich, 1912), whereas in *Acanthias*

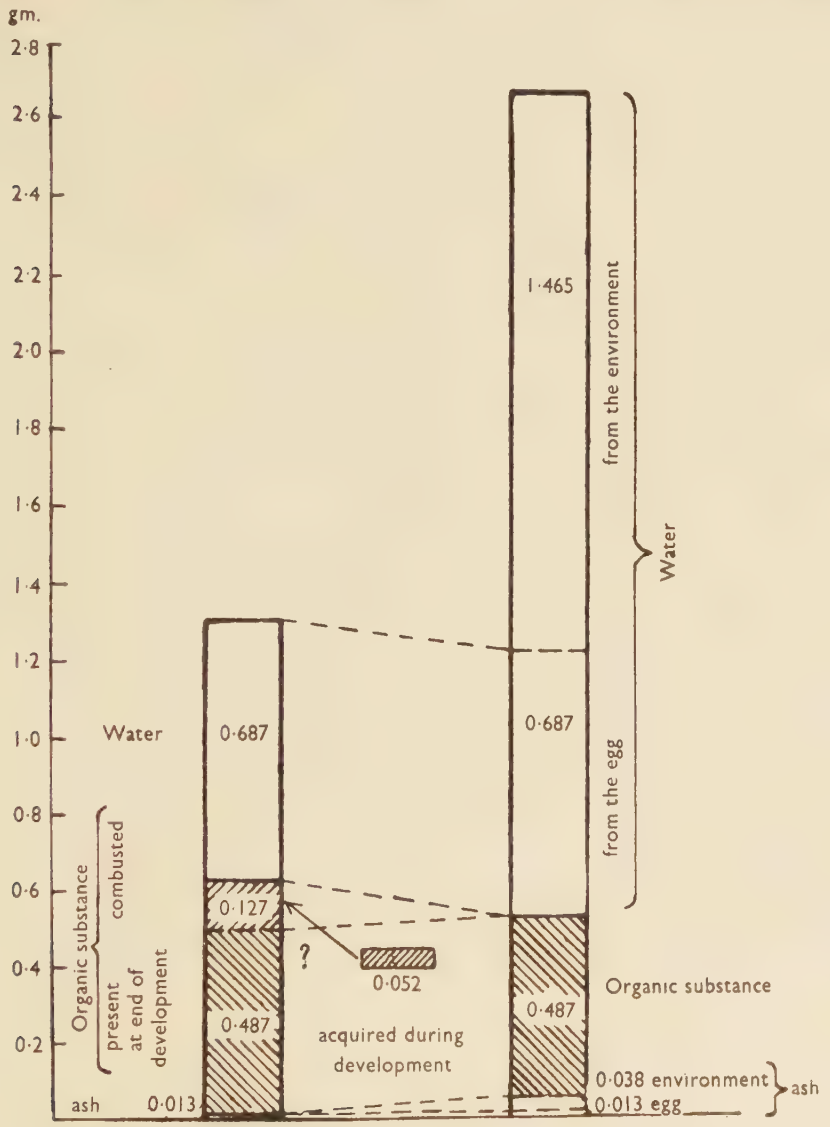

Fig. 1.—Diagram illustrating the differences in composition of the egg of *Scylliorhinus canicula* at the beginning and at the end of development. (Ranzi, 1932 b).

blainvillei, it is less abundant but more concentrated and has a concentration of organic material of about 3 per cent. As the dependence of the embryo upon the maternal organism increases, the uterine fluid becomes turbid, its concentration in organic substances increases and there is an increasing

TABLE 1.—Dependence of eggs on the aquatic environment (Ranzi, 1932 b)

Species	Yolk (wet wt.) gm.	+ External water gm.	+ External ash gm.	= Completed embryo gm.	+ Organic substance combusted gm.
Squid					
Sepia officinalis	1·0	0·78	0·033	1·727	0·086
Dogfishes					
Scylliorhinus canicula	1·0	1·115	0·029	2·047	0·097
Torpedo ocellata	1·0	1·087	0·0138	1·972	0·1288
Torpedo marmorata	1·0	0·993	0·018	1·890	0·1210
Scymnus lichia	1·0	0·43	0·05	1·441	0·0390
Centrophorus granulosus	1·0	0·428	0·279	1·168	0·539

TABLE 2.—Percentage composition of uterine milk in various selachian fishes (Ranzi, 1934)

Species	pH	Na/D	Water	Organic Substance	Fat	Ash
Ovoviviparous						
Torpedo ocellata	7·0	1·34105	96·3	1·2	0·1	2·5
Torpedo marmorata	7·0	1·34105	96·4	1·5	0·1	2·1
Scymnus lichia	5·9	1·34179	95·1	1·6	0·1	3·3
Centrophorus granulosus	–	1·34192	95·1	1·8	–	3·1
Acanthias vulgaris	7·0	1·34313	95·1	2·4	0·3	2·5
Acanthias blainvillei	–	1·34233	95·1	2·8	–	2·1
Galeus canis	–	–	93·8	4·9	trace	1·3
Mustelus vulgaris	6·5	1·34551	93·6	5·1	0·2	1·3
Mustelus antarcticus	–	–	90·9	7·1	–	2·0
Trygon violacea	7·0	–	86·5	13·3	8·2	1·2
Viviparous						
Mustelus laevis	6·2	1·35313	89·4	9·1	0·1	1·5

accumulation of formed elements (leucocytes) in suspension (Table 3). In the sting-ray *Trygon*, for example, the " uterine milk " contains upwards of 13 per cent organic substance, 8 per cent of which is in the form of fat. In addition to this, the negative balance of organic substance caused by combustion is, as Ranzi (1932 b, 1934) and Needham (1942) have pointed out, gradually changed over into a positive balance when the embryo comes from species in which the foetal tissues are brought into closer and closer contact with the maternal organism. For example, in *Centrophorus granulosus* (Table 3) there is still a loss of organic substance by combustion during development, whereas in *Acanthias blainvillei* there is neither a total loss nor a total gain. On the other hand, in the smooth dogfish *Mustelus vulgaris* and others there is gradually increasing accumulation of organic material from the maternal stores, which reaches its maximum in the sting-ray *Trygon violacea*, where the gain in organic substance is some two thousand per cent (Tables 3 and 4). In all these ovoviviparous forms the process is repeated for inorganic substance and water (Tables 5 and 6). Thus, whereas the water intake of the oviparous dogfish *Scylliorhinus canicula* is about 200 per cent of the initial store, in *Mustelus vulgaris* and *Trygon violacea* (both ovoviviparous forms), it is several thousand per cent.

STRUCTURAL ADAPTATIONS ASSOCIATED WITH SELACHIAN OVOVIVIPARITY AND VIVIPARITY

Turning now to the modifications in the uterine wall which accompany the gradually increasing concentrations of organic substances in the uterine fluid, Ranzi (1934) has shown that this differs among the different groups of selachian fishes. In some, the secretion of " uterine milk " (Amoroso, 1952, 1955 a) is accomplished with little or no alteration of the uterine wall, while in others a more or less complete reorganization of the uterine lining occurs. This latter may be smooth and may contain many mucus secreting cells as in *Mustelus vulgaris* or project into longitudinal folds which are invested with a

TABLE 3.—Intake of organic substance by eggs of oviparous, ovoviviparous and viviparous selachian fishes (Ranzi, 1932 b, 1933)

Species	Gestation in months	Weight of egg at the beginning and end of development				Weight of organic substance			
		Egg gm.	Completed embryo gm.	Difference	% Change	Egg gm.	Completed embryo gm.	Difference	% Change
Oviparous									
Scylliorhinus canicula	6	1·314	2·69	+ 1·376	105	0·614	0·487	− 0·127	− 21
Ovoviviparous									
Torpedo ocellata	4	6·78	13·37	+ 6·59	97	3·780	2·91	− 0·87	− 23
Torpedo marmorata	6	14·38	27·28	+ 12·90	90	7·680	5·06	− 2·62	− 34
Scymnus lichia	10	129·53	186·66	+ 57·13	44	62·72	53·50	− 9·22	− 15
*Centrophorus granulosus**	10	308·0	360·0	+ 52·0	17	162·0	74·0	−88·0	− 54
Acanthias blainvillei	9	19·26	38·83	+ 19·57	102	10·68	10·79	+ 0·11	+ 1
Mustelus vulgaris	10	3·928	60·16	+ 56·23	1432	1·956	8·93	+ 6·974	+ 356
Trygon violacea	2	1·9	118·0	+116·10	6105	0·93	16·07	+15·14	+1628
Viviparous									
Mustelus laevis	5	5·535	189·6	+184·1	3326	2·811	32·70	+29·90	+1064

* Combined data of Lo Bianco (1909) and Ranzi (1932 b)

TABLE 4.—Dependence of the eggs of selachian fishes on the uterine environment in ovoviviparity and viviparity (Ranzi, 1932 b)

	Yolk (wet wt.) gm.	+ External water gm.	+ External organic substance gm.	+ External ash gm.	= Completed embryo (wet wt.) gm.
Ovoviviparous					
Acanthias blainvillei	1·0	2·26	0·0013	0·019	3·28
Mustelus vulgaris	1·0	12·18	1·77	0·356	15·31
Trygon violacea	1·0	52·55	8·40	1·08	63·03
Viviparous					
Mustelus laevis	1·0	26·90	5·04	0·948	33·89

stratified epithelium containing few mucus secreting cells (*Acanthias vulgaris*, Pl. 1, fig. 1), or grow into villi (*Torpedo ocelata*, *Pteroplatea micrura*, *Myliobatis nieuhofii*, *Trygon walga*).

In the Batoidea (the skates and rays), *Pteroplatea* develops long villi in the walls of the uterus which enter the spiracle of the embryo and extend down into the oesophagus. These extensions of the mucous membrane of the uterus, to which the term " trophonemata " is applied (Wood-Mason & Alcock, 1891), are beset with tubular glands so that their secretions reach the alimentary canal of the young fish directly. They also probably assist in respiration. In *Torpedo* the villi are more numerous but not so extended. The epithelium is one-layered and there are many glands but no mucus secreting cells ; while in *Trygon* there are as many glands as before, but the epithelium is stratified and the villous condition reaches its climax. These thick-set papillae secrete the uterine milk and there is no organic connection between the offspring and its mother. In *Myliobatis nieuhofii*, Alcock (1890) states that the uterine papillae are less attenuated and the whole uterine mucosa forms a superficial gland.

In *Torpedo* the uterine fluid passes into the digestive tract through the mouth and spiracles. At an early stage the digestive glands of the *Torpedo* embryo are all functional (Pitotti, 1936). Thus, while the uterine fluid is being digested in the stomach, the yolk is being digested in the intestine. The same would appear to be true for *Pteroplatea* as well (Amoroso, 1952).

Yet another method of viviparity is shown by some of the Selachii. In *Lamna cornubica*, Shann (1923) has shown that following the early absorption of the yolk, the nutrient material is derived from the ovary in the form of immature eggs or partly degenerated ovarian tissue which are taken by the oviduct and passed into the uterus where they are swallowed by the embryo. Feeding like this continues for over a year, when the cardiac stomach assumes gigantic proportions and is then called the " yolk stomach ". This pronounced modification of the stomach closely parallels the temporary elongation and expansion of the hind-gut seen in the embryos of anablepid teleost fishes (see below), and it is possible that it provides an important pathway for the absorption of nutritive materials from the parent, although conclusive evidence is lacking.

TABLE 5.—Intake of inorganic substance by eggs of oviparous, ovoviviparous and viviparous selachian fishes (Ranzi, 1932 b)

Species	Gestation in months	Weight of ash at beginning and at end of development			
		Egg gm.	Completed embryo gm.	Difference gm.	% Change
Oviparous					
Scyllíorhinus canicula	6	0·013	0·051	+0·038	292
Ovoviviparous					
Torpedo ocellata	4	0·060	0·154	+0·094	157
Torpedo marmorata	6	0·140	0·40	+0·260	186
Scymnus lichia	10	1·51	8·00	+6·490	430
*Centrophorus granulosus**	10	3·40	12·00	+8·60	253
Acanthias blainvillei	9	0·22	1·76	+1·54	700
Mustelus vulgaris	10	0·05	1·45	+1·40	2800
Trygon violacea	2	0·02	2·07	+2·05	10250
Viviparous					
Mustelus laevis	5	0·069	5·32	+5·25	7609

* Combined data of Lo Bianco (1909) and Ranzi (1932 b)

TABLE 6.—Intake of water by eggs of oviparous, ovoviviparous and viviparous selachian fishes (Ranzi, 1932 b)

Species	Gestation in months	Weight of water at the beginning and end of development			
		Egg gm.	Completed embryo gm.	Difference gm.	% Change
Oviparous					
Scylliorhinus canicula	6	0·687	2·152	+ 1·465	213
Ovoviviparous					
Torpedo ocellata	4	2·94	10·31	+ 7·37	251
Torpedo marmorata	6	6·56	21·85	+ 14·29	218
Scymnus lichia	10	65·30	131·0	+ 55·70	85
*Centrophorus granulosus**	10	142·0	274·0	+132·0	93
Acanthias blainvillei	9	8·36	26·28	+ 17·92	214
Mustelus vulgaris	10	1·922	49·78	+ 47·86	2490
Trygon violacea	2	0·95	99·86	+ 98·91	10412
Viviparous					
Mustelus laevis	5	2·655	151·6	+148·9	5608

* Combined data of Lo Bianco (1909) and Ranzi (1932 b)

These methods of nourishing the embryo resemble to some extent those found in the *Holocephali*, which are oviparous. In this group each egg is enclosed in a horny capsule, but Dean (1906) found that only a small part of the yolk is included in the yolk sac, the remainder breaking up to form a heavy milky fluid which is first absorbed by the long filamentous gills of the embryo, and later ingested by the mouth.

THE SELACHIAN YOLK-SAC PLACENTA

It is, however, with the development of a yolk-sac placenta that viviparity reaches its highest expression in the Selachii (Amoroso, 1952 ; 1955 a). In some species there is a specially modified portion of the yolk sac which comes in contact with the uterine wall and through which food material and oxygen are absorbed from the mother and waste products removed. According to Cole (1944), " it was the Danish anatomist Steno (1673) who was the first after Aristotle, to claim that the connection was a functional placenta, by the activity of whose blood vessels soluble food materials were transferred from the parent to the cavity of the yolk-sac, and thence by its stalk to the gut of the embryo ". It has been described in *Mustelus laevis* (Müller, 1840 ; Ranzi, 1932 b, 1934; ten Cate Hoedemarker, 1933), *Carcharius glaucus* (Müller, 1840 ; Calzoni, 1936), *Carcharius melanopterus* (Alcock, 1890) and in *Scoliodon surrakowah* and *S. palasorrah* (Mahadevan, 1940).

In *Mustelus laevis* the embryos are separated by compartments and in the placental area the uterine wall develops villi which interlock with grooves on the surface of the yolk-sac. At these points the stratified uterine epithelium and the epithelium of the yolk-sac become modified and are greatly reduced in thickness, thus exposing the maternal and foetal capillary blood vessels to the egg membrane. In this way the two circulations are brought surprisingly close to one another at these points and subserve the most direct interchange of substances—especially the gaseous exchange —between the mother and her offspring.

This type of yolk-sac placenta is further evolved in some species of *Scoliodon*. As in other viviparous elasmobranchs, the embryos in *Scoliodon* are lodged in separate compartments of the uterus, the mucosa of which is raised in places and then invaginated in the form of a single long trophonematous cup with a rich vascular supply. Both the yolk-sac and the part of the trophonematous cup, to which it becomes connected, undergo important changes resulting in an almost complete union between the two structures. In most species of *Scoliodon* special structures, the appendicular, are developed on the placental cord. They help in the absorption of the uterine secretions.

An important feature of the yolk-sac of *Scoliodon sarrakowah* is that the cavity of the yolk sac is not filled with yolk granules but with a network of cellular strands saturated with circulating blood, recalling the condition described in *Salpa democratica* (Amoroso, 1955 a). The yolk stalk is likewise obliterated forming a placental cord, so that in the ripe placenta the connection between mother and offspring becomes purely vascular as in mammals.

But notwithstanding the development of placental connections in these viviparous forms, it seems that nourishment of the embryo by means of the elasmobranch embryotrophe (Amoroso, 1952, 1955 a), is so efficient as to exceed the efficiency of the viviparous types, in which the yolk-sac actually invades the uterus (see Tables 3, 4, 5 and 6). Thus in *Mustelus laevis*, the type specimen of selachian viviparity, the uptake of water, ash and organic material, in percentage of the initial stores, is actually rather less than in some of the most successful ovoviviparous types such as *Trygon*. It is to be remembered, however, that there may be many other reasons which have led to the evolution of true viviparity in these fishes. Indeed, Ranzi (1932 b, 1934) pointed out that the speed of development and absorption is much greater in *M. laevis* than either *M. vulgaris* or *M. antarticus*.

MATERNAL LIVER CHANGES IN OVOVIVIPARITY AND VIVIPARITY

This brings us to the question of the reduction of the maternal liver during elasmobranch gestation. Ranzi (1933) noticed that there was a parallelism between the degree of dependence of the embryos on the mother and the degree to which the liver is reduced in size during their development. A gradual fall in maternal liver weight is observed when the embryos come from species in which the foetal tissues are brought into closer and closer contact with the maternal organism. The rise and fall in liver weight appears to be integrated into the selachian endocrine cycle of reproduction (Ranzi, 1935).

According to Ranzi & Zezza (1936) there is no destruction of cells during the rise and fall in liver size, but only a swelling followed by a shrinking, so that as gestation advances, the number of nuclei visible in a single standard section of the maternal liver constantly rises, whereas, the number of lipoid droplets correspondingly falls (see, however, Polimanti, 1912).

As to the nature of these lipoid substances, Andre (1927) established the fact that livers of elasmobranch fishes contain more oil during gestation than when non-gestating. Andre & Canal (1929) showed, moreover, that the liver oil of the young pilgrim shark (*Cetorhinus maximus*) and the embryonic liver of *Centrophorus granulosus*, both contain less saponifiable matter and relatively more cholesterol than in the liver of the parent. Moreover, in both animals the embryo and young fish is richer in neutral fat, and this is especially pronounced in the eggs of *Centrophorus*, where there is as much saponifiable as unsaponifiable matter. Furthermore, the iodine values of the fat of the maternal liver in *Trygon* are much higher than those of the uterine fluid (Stolfi, 1934), which, as previously mentioned, is especially rich in fat. It may, therefore, be concluded that the fatty acids undergo saturation in passing to the embryo, but how or where this takes place remains unknown.

TELEOSTEAN OVOVIVIPARITY AND VIVIPARITY

In the Teleostei, which form by far the largest group of Osteichthyes, reproduction is generally oviparous with external fertilization, large numbers of eggs and spermatozoa being liberated into the water. In a small number of teleost fishes, however, fertilization is internal ; in them the number of eggs

TABLE 7

Order, Family or Species	Type	Fertilization	Gestation	Foetal nourishment and placental structures	Corpora lutea
Siluridae *Trachychoristes*	Oviparous	Ovarian cavity or gonaduct	Eggs immediately discharged into water	Yolk in yolk sac and from aquatic environment	?
ACANTHOPTERI					
Scorpaenidae *Sebastodes*	Oviparous	Follicle zygote retained for long period	Brood remain in ovarian cavity for short period ; thereafter development oviparous	Yolk in yolk sac and from aquatic environment	Present
Blenniidae (*Zoarces viviparus*)	Viviparous	Ovarian cavity	Retained in ovarian cavity till birth	Very little yolk ; ovary furnishes greater part of food ; some embryotrophe from dead embryos ; no supplementary structures ; absorption through skin	Present
Embiotocidae (*Cymatogaster aggregatus*)	Viviparous	Follicle	Hatched into ovarian cavity before segmentation completed. Retained for very long period ; embryos born in very advanced state. Males sexually mature at birth	Secretions ; embryotrophe from desquamated cells ; sperm, dead embryos ; absorption through hypertrophied yolk sac. Supplementary structures for respiration in form of vascular spatulate processes on fins	Present

CYPRINODONTES					
Poeciliidae (*Xiphophorus helleri*)	Oviparous	Follicle	Follicular for entire gestation ; high degree superfoetation ; embryos very immature at birth ; gonads in indifferent stage	Yolk provides most of intra-ovarian food, not completely absorbed at birth ; " neck " strap forms supplementary structure for absorption of secretions	?
CYPRINODONTES					
Poeciliidae (*Heterandria formosa*)	Viviparous	Follicle	Follicular for entire gestation ; high degree of superfoetation	Ovarian secretions ; "neck" strap and follicular pseudo placenta	?
Anablepidae (*Anableps anableps*)	Viviparous	Follicle	Follicular for most of gestation ; short time in ovarian cavity	Ovarian secretions ; yolk sac hypertrophied and is principal organ of absorption ; enlargement of gut forms intestinal pseudo placenta	Present
Goodeidae (*Ataenobius toweri*) (*Goodea bilineata*) (*Zoogonecticus cuitzooensis*) (*Lermicthys multiradiatus*)	Ovoviviparous with superimposed viviparity	Follicle	Ovarian cavity for entire gestation ; brood in advanced state of development at birth ; high degree of mortality	Ovarian secretions ; embryotrophe from stored sperm and dead embryos ; trophotaeniae (foetal) absent in *Ataenobius toweri*, poorly developed and voluminous in *Zoogonecticus cuitzooensis* and *Lermichthys multiradiatus*	Present
Jenynsiidae	Ovoviviparous with superimposed viviparity	Follicle	Ovarian cavity for entire gestation ; brood in advanced state of development at birth ; high degree of mortality	As for Goodeidae but ovarian fluids imbibed directly by mouth ; trophonemata (maternal) in the form of ovarian flaps constitute a branchial pseudo placenta	Present

has been secondarily reduced, and the developing young are retained not in the gonaduct but within the ovarian cavity or within the follicle itself (Turner, 1933, 1940 a, b, c, d).

From the multitude of varied phenomena that Turner has recorded, two salient facts stand out. First, viviparity is, with few exceptions, concentrated in two orders, the Cyprinodontes (families, Poeciliidae, Anablepidae, Goodeidae and Jenynsiidae) and the Acanthopteri (families, Embiotocidae, Scorpaenidae, Blenniidae, and Zoarcidae), being especially well developed in the order Cyprinodontes in which all genera and species of the four families have viviparity more or less well developed. Secondly, all grades of maternal dependence occur in teleosts as they do in selachians. These range from little or no alteration of the oviparous types of structures (e.g. the guppy, *Lebistes reticulatus*), to conditions in which the embryos respire and are nourished by the maternal organism (Pl. 1, fig. 2).

The first step toward viviparity is intromission of sperm into the gonaduct. In order, therefore, that fertilization might be accomplished, it is necessary that the spermatozoa be able to live, for short periods at least, in the cavity of the ovary itself.

The ability of the spermatozoa to remain alive within the ovary is partly a function of the ovary itself. In some cases of teleostean viviparity it is the epithelium of the ovarian cavity which assumes a trophic function, enabling the immobilized sperm to be kept alive for varying periods of time. In others e.g. *Xiphophorus* (Turner, 1933), and *Neotoca bilineata* (Wiedersheim, 1899 ; Mendoza, 1943), it is the corpora lutea derived from the granulosa and theca interna of the discharged follicles which produce a copious secretion for the nourishment of the sperm and of the young if fertilization takes place.

Among viviparous teleosts, fertilization may occur while the egg is still retained within the follicle or after its extrusion into the ovarian lumen (Table 7). As an initial step in the evolution of teleostean viviparity it could be presumed, therefore, that ovulation would have taken place into the ovarian cavity or into the gonaduct prior to fertilization, and that the zygote was extruded from the gonaduct before embryonic development was far advanced. Examples of this type of viviparity are to be found in *Trachychoristes*, a Brazilian catfish, and in the phallostethid fishes of the East Indies (Turner, 1947).

Another case of viviparity, involving fertilization of the egg after it had been evacuated from the follicle into the ovarian cavity appears to have occurred in the viviparous blenny of European seas *Zoarces viviparus* (Stuhlman, 1887 ; Wiedersheim, 1899 ; Turner, 1947). In this brotulid fish, the embryos are retained for long periods of time in the ovarian cavity and the evidence points to the occurrence of ovulation prior to fertilization.

A more advanced step in the development of viviparity is the fertilization of the egg within the ovarian follicle prior to ovulation. Presumably this is dependent upon the ability of the spermatozoa to remain alive within the ovary for long periods of time. Moreover, when it is realised that the ovarian epithelium is in contact with the follicular wall just prior to fertilization and that only a single layer of flattened attenuated cells of the medulla separates the

spermatozoa from the columnar cells of the follicle, it is easy to see how fertilization can be accomplished by the spermatozoa that are embedded in the ovarian epithelium. In *Cymatogaster aggregatus* (Turner, 1938 b) and in *Xiphophorus helleri* (Turner, 1933), the sperm are said to remain alive for months in the ovarian cavity serving to fertilize eggs for several succeeding broods, whilst in *Heterandria formosa*, live sperm have been found in the ovary ten months after the female has had a single contact with the male (Turner, 1947).

Evidence is available to show that fertilization within a follicle can and does occur. In *Xiphophorus* (Bailey, 1933) *Anableps* (Turner, 1938 a), *Cymatogaster* (Eigenmann, 1892 c ; Turner, 1938 b) *Jenynsia* (Turner, 1940 d), *Sebastes marinus* (Williamson, 1910) and in goodeid fishes (Turner, 1933 ; Mendoza, 1943), eggs are fertilized while still enclosed within the follicle and later are discharged into the ovarian lumen at different stages of development. However, in *Cymatogaster*, *Sebastes murinus* and in *Neotoca bilineata*, fertilization and the expulsion of the egg must occur in such rapid succession that it is difficult to separate the two events. Similarly, the period of retention in the follicle is very brief in the goodeid fishes, the embiotocid fishes, and some of the brotulids. In these the follicular wall breaks down and the embryos, while still in the segmentation stages, are discharged into the lumen of the ovary where many complete their development, being nourished with embryotrophe derived from the ovarian secretions and disintegrating embryos. On the other hand, in forms such as *Anableps* and *Xiphophorus*, while fertilization occurs within the follicle, the embryos are not released until a short time preceding birth, so that ovulation and birth are almost synchronous events.

In embryos developing within the ovarian cavity conditions are remarkably different from those in follicular gestation. The young are not separated from one another as they are in follicular gestation, but are all contained in a space lined by the epithelium of the ovarian cavity and are bathed by fluids secreted by the epithelium. In *Sebastodes* the retention in the ovarian cavity is short, the brood consisting of thousands of embryos being discharged into the water where they continue their development in the manner characteristic of oviparous fishes. On the contrary, the embryos are retained longest in some of the surf perches (Embiotocidae), the males, at least, being sexually mature when born.

SUPERFOETATION

Within the family Poeciliidae there are a number of species having follicular gestation that are distinguished from the typical poeciliids by super-foetation. In this condition, two (e.g. *Poeciliopsis*) and in some extreme instances (e.g. *Aulophallus*, *Heterandria formosa*), as many as eight or nine small broods of young, representing each a different stage of development, may be found within the ovary at the same time ; in these the modifications in the embryo and in the ovarian follicle are like those of typical poeciliids.

Effective superfoetation, as far as is known (Turner, 1937 a, 1947), occurs only in the poeciliid fishes of the order of Cyprinodontes and in this family it has arisen twice independently. Turner (1947) suggests that the condition

has been evolved by the shortening of the period required for developing and maturing the egg so that a second group of eggs reaches maturity some time before the previous brood of embryos is evacuated from the follicle, and the eggs of the second group are fertilized, each within its follicle.

STRUCTURAL BASIS FOR OVOVIVIPARITY AND VIVIPARITY IN TELEOST FISHES

Unlike the higher vertebrates almost all viviparous teleosts have a single median ovary which is attached dorsally to the pleuro-peritoneal membrane and ventrally to the mesogaster. Only in forms such as *Sebastodes rubrovinctus* (Eigenmann, 1892 a) is the ovary double, and only in a few forms, such as *Dermogenys pusillus* (Peters, 1865), *Lucifuga subterraneous* and *Stygicola dentata* (Lane, 1903) is the ovary partially fused. But so far as is known, all viviparous teleosts have the single gonaduct, even in forms that have the double or partially fused gonads.

The ovary of teleost fishes may be a solid or a hollow organ. In the former case the eggs merely rupture the wall of the gonad, fall into the coelom and then escape to the exterior through abdominal pores. In the latter type which is characteristic of viviparous forms, the eggs never reach the coelom but make their way directly from the ovary through the gonaduct, which, it is to be observed, is probably not homologous with the oviduct of other animals. As is true of all viviparous teleost ovaries, the gonad is continuous with the short gonaduct which extends posteriorly as a direct continuation of the muscular wall of the ovary, to open externally in the vent.

The structural relations within the ovary are fundamentally alike in all strictly viviparous teleosts, namely an external sac, to which are attached, in sundry fashions, lobulated ovigerous folds bearing the germinal tissue. The internal surface of the ovarian wall is characterized, in turn, by low longitudinal folds which likewise project into the ovarian cavity. The tissues of the ovary are few. A continuous internal epithelium lines the ovarian cavity, completely investing the ovigerous folds, the ovarian wall and the median septum where this latter is present. A loose richly vascularized subepithelial stroma of connective tissue forms the bulk of the ovigerous folds and the septum, and is, in its turn, limited externally by a layer of circular muscle continuous with that of the gonaduct.

In live-bearing bony fishes, early angiogenesis occurs within the body alone. The yolk-sac becomes vascularized later, by veins growing out from the embryonic body. When the gills are still short, a fine plexus of vessels, constituting the portal capillary network develops over the surface of the yolk sac and the expanded portion of the pericardial sac. Through this elaborate portal network must pass all the blood that has circulated through the body before it enters the heart. It must be emphasized, however, that this portal circulation is not the vitelline circulation of the other vertebrates, which in some viviparous forms plays an important part in the vascularization of the yolk-sac or chorio-vitelline placenta.

In many species of viviparous teleosts a collar-shaped extension, the " neck-strap " (Turner, 1940 a), rises from the upper margins of yolk-sac and

pericardial sac ; in some it envelops the entire head. This formation closely resembles the chorio-amniotic fold of the higher vertebrates, except for its peculiar, rich venous blood supply. The parallelism between the enveloping folds of the pericardium in some poeciliids (e.g. *Heterandria formosa*) and the amniotic folds in amniotes is striking. In both there is an hypertrophy of the heart producing an extra-embryonic coelom (extra-embryonic pericardial cavity in *H. formosa*, Turner 1940 a).

ADAPTATIONS FOR VIVIPARITY IN EMBRYOS AND OVARIES OF TELEOST FISHES

As previously mentioned, teleost fishes constitute yet another case of apparent succession of adaptations, associated with the birth of living young, which range from little or no alteration of the oviparous types of structures (e.g. the guppy, *Lebistes reticulatus*) to conditions in which the yolk supply is reduced and a series of temporary, but unique, structures occur (Pl. 1, fig. 2 ; Pl. 2, figs. 3, 4, 5 and 6). The latter are associated with the exchange of materials between embryo and ovary ; this is true of embryos residing within ovarian follicles or ovarian cavities. Conversely, in follicular gestation and in gestation within the ovarian cavity, there are some simple modifications of structure in cases in which the embryos have an adequate supply for their periods of gestation.

FOLLICULAR GESTATION

Poeciliidae and Anablepiidae

In simple follicular gestation (e.g. *Mollienisia latipinna*, *Xiphophorus helleri*, *Lebistes reticulatus*) the embryos are provided with a supply of yolk adequate for their period of gestation. Moreover, the assumption of a viviparous habit by these typical poeciliids has been accompanied by very little modification of structure in the embryo and presumably of function also (Pl. 1, fig. 2). In *Xiphophorus*, the ovarian follicle builds up within each oocyte a large yolk mass which is to be almost the sole means of nourishment for the future embryo up to the time of its birth (Turner, 1933). A recent study by Bailey (1933) indicates, however, that the embryo depends not only upon its yolk but also receives materials, which contribute to its growth, from the capillaries of the ovarian follicle in which it is retained.

The embryo remains within the ovarian follicle until hatched, hatching being defined as a rupture of the vitelline membrane and the wall of the ovarian follicle so as to free the embryo in the intraovarian cavity. The immature embryo is then born immediately. At birth the yolk mass is not entirely absorbed ; the portal circulation upon the yolk-sac and the pericardial sac being much like that found in oviparous fishes.

Follicular gestation involving embryos with small yolk masses is accompanied by the development of a number of extreme and temporary modifications in both embryos and ovaries. In *Heterandria formosa* (Turner, 1940 a ; Fraser & Renton, 1940 ; Scrimshaw, 1944, 1945), the embryos are retained within the ovarian follicles. The yolk mass is reduced and the pericardial sac, upon which an extensive portal capillary network develops, become

enormously expanded so as to enclose the head. The follicular wall becomes very thin, highly vascular, and closely applied to all the exposed external surfaces of the embryo. Where the two are in contact, the follicular epithelium is thrown into low folds or villi, and since the latter have likewise developed an extensive capillary bed, an association between the two circulations, comparable to the mammalian epithelio-chorial placenta is formed. For the complex composed of the follicular wall with its villi, the follicular space and the adjacent vessels of the portal system covering the belly sac, Turner (1940 a) has proposed the term "*follicular pseudo-placenta*".

The expanded pericardium surrounding the embryos in *Heterandria formosa* (Fraser & Renton, 1940) poses the question whether the amnion arose as a cushion in viviparity, or as an aquarium in terrestrial life.

In anablepid fishes (e.g. *Anableps anableps*), on the other hand, there is a great expansion of the pericardial and peritoneal sacs and a pseudo-placenta is formed by an expansion of the middle portion of the intestine and the development of vascular bulbs on the portal vein tributaries. In another species *Anableps dowei*, it is the posterior portion of the intestine that undergoes temporary elongation ; in both *Anableps anableps* and *Anableps dowei* long internal villi are developed in the gut. On the inner surface of the follicular wall, great numbers of follicular villi are formed and a loose placental relationship is thus established between the vascular wall of the follicle and the vascular surfaces of the pericardial and the peritoneal sacs of the embryo (Pl. 2, fig. 3).

Comparing the adaptations for viviparity in the two families Poeciliidae and Anablepiidae, it is clear that there are many points of resemblance and many points of difference. Thus, while the embryos of different genera and species of the Poeciliidae have developed different degrees of specialization for viviparity, those of the Anablepiidae all show a degree of specialization beyond that developed in any of the poeciliids. For example, it is clear that the expansion of the pericardial sac in the embryos of the two viviparous families is a parallel development. On the other hand, there is nothing in the poeciliids to compare to the vascular bulbs in the belly-sac portal system of the anablepids. Similarly, the expansion and enlargement of the gut in the Anablepiidae has no parallel in the Poeciliidae, whereas, the expansion of the coelomic sac in the Poeciliidae and the development of the pseudo-amnion and pseudo-chorion in *Heterandria formosa* (Poeciliidae) have no parallel in the anablepid embryos.

GESTATION WITHIN THE OVARIAN CAVITY

Goodeidae and Jenynsiidae

Whereas in the Poeciliidae and the Anablepidae, the embryos are retained during gestation within modified ovarian follicles, in the goodeid and jenynsiid fishes the embryos are discharged from the ovarian follicles into the ovarian cavity where they undergo their development. All the instances, with one possible exception, in which the embryos are retained in the ovarian cavity, have arisen from types which earlier in their evolution retained the embryos for some time within the ovarian follicle (Turner, 1947). The expanded and

vascular peritoneal and pericardial sacs characteristic of the follicular type of gestation are present temporarily and are then quickly replaced by other new structures upon which the embryo depends later, while enclosed within the ovarian cavity.

In *Jenynsia* (Scott, 1928) the egg develops with a very small yolk mass and degeneration of eggs is widespread. The impossibility of all the embryos receiving sufficient nutrition has resulted in all cases in a high intra-ovarian mortality and an absorption of the dead embryos by the surviving ones.

The follicular wall ruptures about the time segmentation is completed and the embryo is then free in the intra-ovarian cavity. Here it depends for nutrition upon the secretions of the ovary, upon stored sperm and upon " intra-ovarian cannibalism ". This material, together with material derived from the autolysis of the ovarian tissues, enriches the ovarian liquor and is directly utilized by the surviving young.

At first, the ovarian epithelium becomes secretory ; it then regresses completely, and in late gestation is replaced by a series of highly vascular folds which partially fill the ovarian cavity and come in contact with the gills, sometimes even entering one of the opercular clefts (Pl. 2, fig. 4). In the stroma the blood vessels become relatively quite numerous and many of the minor branches, especially in *Jenynsia lineata*, are said to enter the overlying epithelium where they pursue an intra-epithelial course.

This arrangement in the jenynsiid fishes may be looked upon as a potential " *branchial placenta* ", for here the epithelium of the gills and the ovarian epithelium are closely approximated, and when it is realized that over a considerable area only the single layer of branchial epithelium plus a thin cytoplasmic frond of the ovarian epithelium separates the two blood streams, the intra-epithelial capillary plexuses acquire greater significance as a means of exchange between mother and offspring.

In the Goodeidae a different device is utilized for furnishing the embryos with nutrients for further growth while they are retained within the ovarian cavity. Extensive rosette-shaped proctodeal processes, the " trophotaeniae " are developed (Turner, 1937 b, 1940 c). These nutritive processes become well supplied with blood vessels and wide tissue spaces, and are covered in some genera by a compact cuboidal epithelium. They are believed to supplement the respiratory functions of the gills by replacing, in some cases (e.g. *Zoogonecticus cuitzoensis*), the pericardial sac functionally (Pl. 2, fig. 5). Nutritive substances are also apparently absorbed by the gut from the fluids drawn into the pharynx and swallowed—a device employed to some extent by the embryos of all viviparous cyprinodonts. The embryos are born in an advanced stage in which the gonads are differentiated into recognizable ovaries and spermaries.

The goodeids, as a group, have gone far in the diminution of the yolk sac and in this regard may be considered as more specialized than any of the poeciliids, except *Heterandria formosa*, *Aulophallus* and the highly-specialized *Poeciliopsinae*. However, in its adaptation for viviparity the primitive and largest of the goodeids, *Ataeniobius toweri* differs but little from the viviparous cyprinodont embryo and the less specialized poeciliid embryo. The most

characteristic feature—the trophotaeniae—of the embryo is lacking (Turner, 1940 c). On the other hand, in *Lermichthys multiradiatus*, in which the trophotaeniae are developed at such an early stage that the function of respiration of the pericardial sac is taken over before the sac has fully expanded, the trophotaeniae become quite voluminous and probably play an important role in the respiratory functioning of the embryo.

Blenniidae and Embiotocidae

In the brotulid fish, *Zoarces viviparus* (Stuhlmann, 1887) the egg leaves the ovarian follicle before fertilization and is fertilized while lying within the ovarian cavity. There are no special supplementary structures for securing nutrition and oxygen. Absorption takes place through the skin, and later nutritive substances in the form of ovarian secretions, desquamated ovarian cells, and other embryos, are taken by mouth and digested. An enlargement of the posterior intestine in this fish has a parallel in the similar enlargement in the posterior portion of the intestine of certain embiotocid embryos. In these latter creatures (e.g. *Cymatogaster aggregatus*) one of the most remarkable of the temporary structures that are developed is a series of flattened vascular extensions of the soft tissues between the ends of the rays in the vertical fins. These vascular protrusions are assumed to facilitate respiration.

Ovarian changes. Turning now to the ovarian changes, there can be little doubt that the ovary of some goodeid fishes (e.g. *Neotoca bilineata*) undergoes very definite cyclical modifications during gestation (Mendoza, 1938, 1943). But, there is a strong possibility that the somatic cycle does not arise solely in response to the presence of embryos, but is a cyclic activation which may even be present in the virgin condition. This activation may now be very precise in its rhythmic appearance ; indeed it would be very surprising if it were not, for it is likely that, judged from an evolutionary point of view, the assumption of the completely viviparous condition by this group of teleosts is quite recent.

The histological changes which characterize the ovary during gestation are described for *Jenynsia lineata* (Scott, 1928 ; Siccardi, 1940), *Xiphophorus helleri* (Bailey, 1933), *Neotoca bilineata* (Turner, 1933, Mendoza, 1940) and *Cymatogaster aggregatus* (Turner, 1938 b). When the embryos are first evacuated into the ovarian cavity, the cells lining the cavity increase in height and in secretory activity. At the same time, the connective tissue of the ovary becomes swollen and free cells, which Mendoza (1940) believes to be a counterpart of the mammalian decidual cells, appear in the connective tissue spaces. The presence of lymph spaces in the ovarian tissues of viviparous teleosts has also been reported in *Zoarces viviparus* (Stuhlmann, 1887) ; *Stygicola dentata* and *Lucifuga subterraneous* (Lane, 1903) ; and in *Cymatogaster aggregatus* (Eigenmann, 1892 b ; Turner, 1938 b). It has been suggested and assumed that the ovarian fluid must subserve important functions of nutrition and respiration. Actually, this assumption is not the result of chemical analysis, but is largely a logical one based on the necessity for such a phenomenon.

Somewhat later the vascularity of the ovary increases and a rich capillary network develops just beneath the epithelial lining of the ovarian cavity. Thereafter, there is a general shrinking of the connective tissue and when the embryos are born, the ovarian tissues involute. Mendoza (1940) sees in all the foregoing changes a remarkable parallel between a viviparous teleost, such as *Neotoca* and a placental mammal. " It is indeed unique," he states, " that two forms taxonomically as separated as are the teleosts and mammals, should become adapted in such a similar manner in response to a similar condition."

DEPENDENCE OF TELEOST EMBRYOS ON THE ENVIRONMENT IN OVOVIVIPARITY AND VIVIPARITY

Finally we come to a consideration of the dependence of the teleost embryo upon the environment. Unfortunately, few biochemical investigations have yet been made from which we can draw any conclusions about the rôle of maternal nutrition in such cases, and most of the physiological interpretations are inferential.

It is likely that the first serious attempt to analyze the ovarian fluid in a viviparous ovary was made by Blake in 1867. He concluded that an "albumin compound with fats, phosphates and iron in the ovarian fluids of embiotocid fishes " was probably present. The brotulid fish *Zoarces viviparus* is said to secrete a very fatty and mucous fluid into the ovarian cavity and Stuhlmann's analysis (1887), an early one of its kind, describes this fluid as " rich in nutritive fluids ", which places it in a position analagous to *Trygon violacea*, in the elasmobranch series. On the other hand, Leiner (1936, 1937) showed that the embryo of *Hippocampus brevirostris*, during its development takes up water and inorganic substances, but not organic substances, from the male organism carrying it. It compares, therefore, with *Scylliorhinus canicula* among the elasmobranchs (Table 1). It is also interesting and perhaps significant in view of what happens in elasmobranchs that in *Gambusia holbrooki*, Remotti (1930, 1935) observed a diminution of liver weight during gestation.

Among viviparous teleost fishes, there is little exact information on the transfer of materials from parent to offspring and vice versa. Hsiao (1940) showed, however, that in the deep sea teleost *Sebastes marinus*, although the eggs are retained in the ovarian cavity, there is a pronounced fall in the dry weight of the developing egg, indicating that there is little, or no, contributions from maternal sources. On the other hand, Scrimshaw (1940) states that whereas the egg in *Heterandria formosa* is only 0·39 mm. in diameter at fertilization, the dry weight of the egg increases from 0·017 mgm. to nearly 10 mgm. during follicular gestation. Moreover, in *H. formosa* there is a great temporary enlargement of the urinary bladder during gestation, and it is possible that urinary wastes are stored in it as they are in the allantoic sacs of some of the higher vertebrates (Turner, 1947). In this remarkable fish, conditions seem to be almost mammalian. The embryo increases in volume many times while lying within the follicular cavity, and in the absence of any considerable quantity of yolk must secure the material necessary for growth from the ovary

of the parent. It is clear, moreover, that the absorption of nutritional materials by these viviparous cyprinodonts must be assisted by the unique, but temporary structures that occur in the embryos only during the gestation period (Pl. 2, figs. 3, 4, 5 and 6).

This assumption is not, however, the result of physiological tests, but is largely inferential and based on the necessity for such physiological adjustments. Accordingly until biochemical correlations have been made on the kind and extent of interchange between offspring and parent in teleost fishes, comparable to those of Ranzi (1932 a, b, c ; 1933 ; 1934), a positive interpretation of the functions of these extraordinary structures must be deferred.

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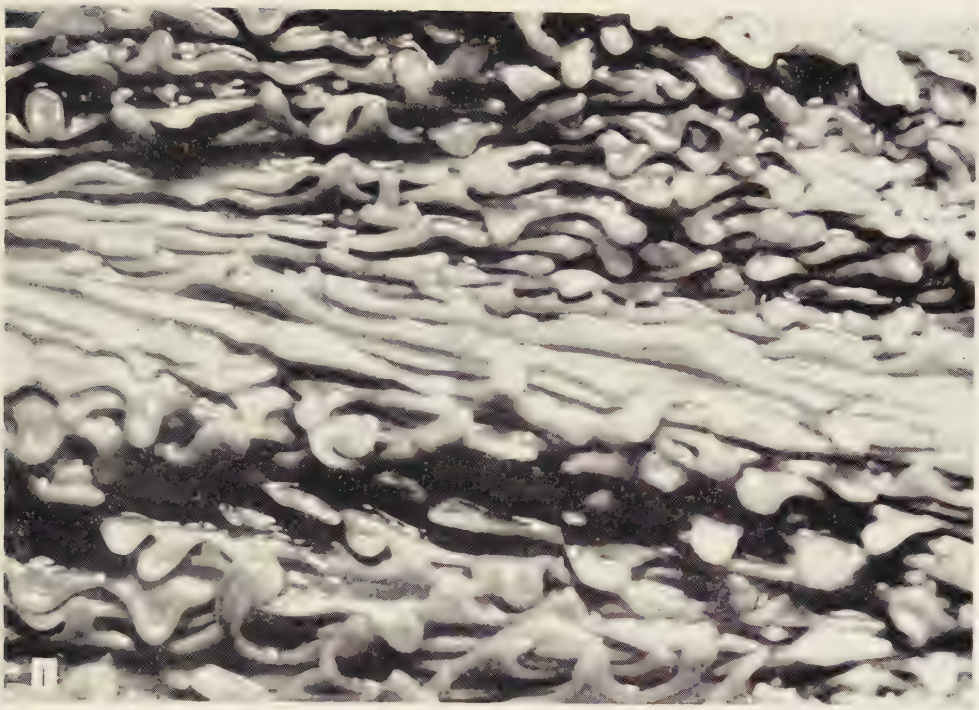

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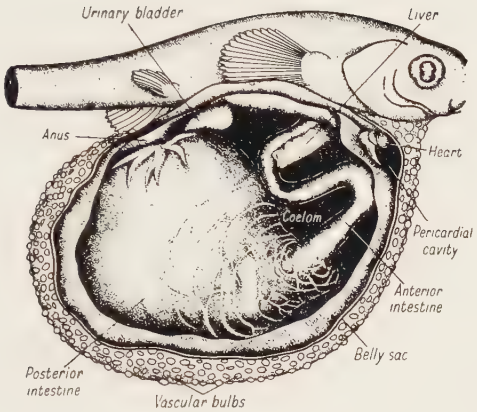

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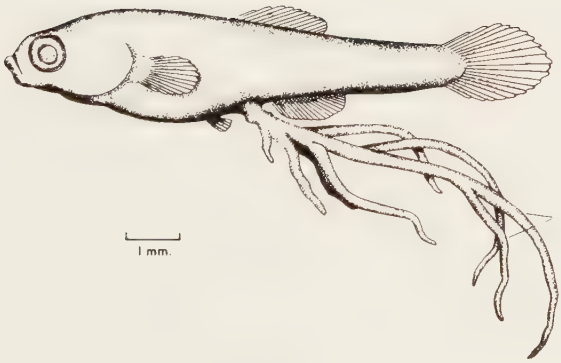

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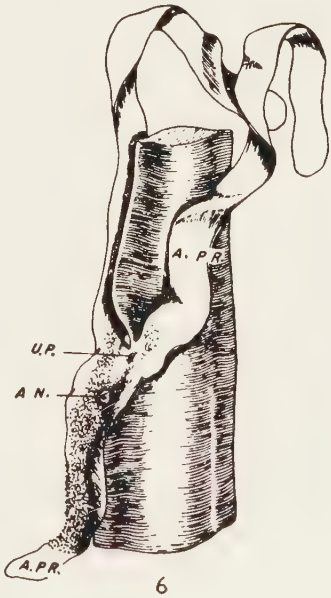

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EXPLANATION OF THE PLATES

PLATE 1.

Fig. 1.—Surface view of the interior of the pregnant uterus of *Acanthias vulgaris*, showing longitudinal folds and papillae × 5.

,, 2.—Section of gravid ovary of *Lebistes reticulatus* with embryos in advanced stage of development.

PLATE 2.

Fig. 3.—Partially dissected embryo of *Anableps dowei*, showing relations of pericardial cavity, coelomic cavity and expanded posterior intestine. (Redrawn from Turner.)

,, 4.—Late stage in the development of *Jenynsia lineata*, showing the attachment of the embryo to ovarian flap. The ovarian tissue extends through the opercular opening into the pharyngeal cavity and a small portion protrudes through the mouth. (Redrawn from Turner.)

,, 5.—Embryo of *Zoogonecticus cuitzooensis*, showing absorptive trophotaeniae. Reprinted by permission of C. L. Turner, *J. Morph.* **67** : 271 (1940).

,, 6.—Posterior part of an unborn embryo of *Parabrotula*, a deep sea fish. The ribbon-shaped membranes of the processes absorb materials from the fluids of the ovarian cavity. Reprinted by permission of C. L. Turner, *Sci. Mon., Lond.* **65** : 508.

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